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1. Shapiro, S., Weiner, M., et al.; *Am. Heart J.* **40**:766 (Nov.) 1950

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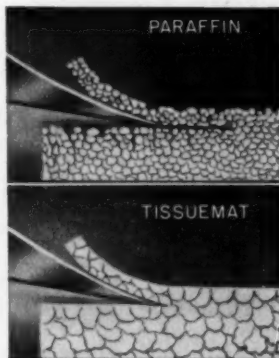
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### BIBLIOGRAPHY

- Joslin, E. P. et al: Treatment of Diabetes Mellitus—8 Ed., Phila., Lea & Febiger, 1946—P. 241, 247.
- Lowsley, O. S. & Kirwin, T. J.: Clinical Urology—Vol. 1, 2 Ed., Balt., Williams & Wilkins, 1944—P. 31.
- Duncan, G. G.: Diseases of Metabolism—2 Ed., Phila., W. B. Saunders Co., 1947—P. 735, 736, 737.
- Stanley, Phyllis: The American Journal of Medical Technology—Vol. 6, No. 6, Nov., 1940 and Vol. 9, No. 1, Jan., 1943.

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## ARTERITIS, CARDITIS, GLOMERULONEPHRITIS AND BILATERAL RENAL CORTICAL NECROSIS INDUCED IN RABBITS

By Injection of Horse Serum or Bovine Gamma Globulin Combined with Killed  
Group A Streptococci or Freund's Adjuvant

ROBERT H. MORE, M.D.

AND

SIDNEY D. KOBERNICK, M.D.

MONTREAL, CANADA

IT HAS been reported on numerous occasions that lesions can be produced in the hearts of animals by rendering them hypersensitive to foreign serum proteins.<sup>1</sup> These lesions have been compared to the cardiac lesions of human rheumatic fever. Experimental lesions have also been seen in the arteries of sensitized animals which resemble the lesions of human periarteritis nodosa<sup>2</sup> and in the kidneys bearing a close resemblance in morphological structure to the lesions of human glomerulonephritis.<sup>3</sup>

The majority of those who have reported the rheumatic-like lesions of the heart have produced them in rabbits by injecting normal horse serum<sup>4</sup> or its

Dr. Kobernick is a Fellow of the Life Insurance Medical Research Fund.

From the Department of Pathology, Pathological Institute, McGill University.

This investigation was supported by a Grant-in-Aid from the National Research Council of Canada.

1. (a) Klinge, F.: *Beitr. z. path. Anat. u. z. allg. Path.* **83**:185, 1930. (b) Vaubel, E.: *Ibid.* **89**:374, 1932. (c) Rich, A. R., and Gregory, J. E.: *Bull. Johns Hopkins Hosp.* **73**:239, 1943; (d) **75**:115, 1944. (e) Bruun, E.: *Virchows Arch. f. path. Anat.* **303**:524, 1939. (f) Apitz, K.: *Ibid.* **289**:46, 1933. (g) More, R. H.; Waugh, D., and Kobernick, S. D.: *J. Exper. Med.* **89**:555, 1949. (h) More, R. H., and McLean, C. R.: *Am. J. Path.* **25**:413, 1949. (i) McKeown, E. F.: *J. Path. & Bact.* **59**:547, 1947. (j) Junghans, E.: *Beitr. z. path. Anat. u. z. allg. Path.* **92**:467, 1934. (k) Ehrich, W. E.; Seifter, J., and Forman, C.: *J. Exper. Med.* **89**:23, 1949. (l) von Hawn, C. and Janeway, C. A.: *Ibid.* **85**:571, 1947. (m) Wissler, R. W.; Smull, K., and Lesh, J. B.: *Ibid.* **90**:577, 1949. (n) Hopps, H. C., and Wissler, R. W.: *J. Lab. & Clin. Med.* **31**:939, 1946.

2. (a) Rich, A. R., and Gregory, J. E.: *Bull. Johns Hopkins Hosp.* **72**:65, 1943. (b) Fox, R. A., and Jones, L. R.: *Proc. Soc. Exper. Biol. & Med.* **55**:294, 1944. (c) Knepper, R., and Waaler, G.: *Virchows Arch. f. path. Anat.* **294**:587, 1935. (d) Masugi, M., and Sato, Y.: *Ibid.* **293**:615, 1934. (e) Klinge.<sup>1a</sup> (f) Vaubel.<sup>1b</sup> (g) Rich and Gregory.<sup>1c,d</sup> (h) Bruun.<sup>1e</sup> (i) Apitz.<sup>1f</sup> (j) More and others.<sup>1g</sup> (k) More and McLean.<sup>1h</sup> (l) McKeown.<sup>1i</sup> (m) Junghans.<sup>1j</sup> (n) Ehrich and others.<sup>1k</sup> (o) von Hawn and Janeway.<sup>1l</sup> (p) Wissler and others.<sup>1m</sup> (q) Hopps and Wissler.<sup>1n</sup>

3. (a) More, R. H., and Waugh, D.: *J. Exper. Med.* **89**:514, 1949. (b) von Hawn and Janeway.<sup>1l</sup> (c) Rich and Gregory.<sup>2a</sup> (d) Hopps and Wissler.<sup>1n</sup> (e) Masugi and Sato.<sup>2d</sup>

4. Klinge.<sup>1a</sup> Vaubel.<sup>1b</sup> Rich and Gregory.<sup>1c,d</sup> Bruun.<sup>1e</sup> Apitz.<sup>1f</sup> More and McLean.<sup>1h</sup> McKeown.<sup>1i</sup> Junghans.<sup>1j</sup> Ehrich and others.<sup>1k</sup> Hopps and Wissler.<sup>1n</sup>

fractions<sup>1m</sup> intravenously; other antigenic substances<sup>5</sup> have been employed in other species.<sup>6</sup> It has been previously pointed out that the cardiac lesions hitherto reported as resulting from the administration of serum have some features in common with the lesions of human rheumatic carditis but that certain features, such as fibrinoid necrosis, a focal interstitial location in the myocardium, typical Aschoff cells and hyaline valvular vegetations, were notably absent.<sup>7</sup> It has also been pointed out<sup>8</sup> that many of the myocardial lesions illustrated in published reports<sup>9</sup> could not be differentiated from lesions occurring in normal animals<sup>10</sup> or those the subject of epizootic infection.<sup>11</sup> Furthermore, in most of the reports in the literature, serum-induced myocardial lesions other than those which can be regarded as spontaneous are so closely related to arteries that are the seat of necrotizing that it is more logical to regard them as part of the latter lesion than as myocardial lesions of the type seen in human rheumatic fever. On the other hand, the lesions of serum-treated rabbits have been compared<sup>12, 13</sup> to the lesions seen in the heart in fatal human cases of serum sickness<sup>12</sup> or of sulfonamide allergy.<sup>13</sup>

If the lesions of serum-treated animals were indeed due to the hypersensitive state induced in the animals, and if the lesions seen in the experimental animals were only quantitatively different from those of human rheumatic fever, it appeared logical to think that one might obviate the differences by enhancing the degree to which the animals were rendered hypersensitive to the serum antigens, horse serum and bovine gamma globulin. Such a possibility was supported by the fact that Freund's adjuvant, which is known to increase hypersensitivity when combined with the antigens,<sup>14</sup> also enhances the tissue lesions produced by injection of brain extracts.<sup>15</sup> However, if the difference between the experimental and human lesions was a qualitative one, the mode of enhancing the hypersensitive state by incorporating the serum antigens in Freund's adjuvant also appeared to offer the possibility of a qualitative alteration of the lesions, since it has been reported that the type of hypersensitivity produced with horse serum was changed from the foreign protein

5. (a) Moore, F. J.; Ridge, G. K.; Huntington, R. W.; Hall, E. M.; Griffith, G. C., and Knowles, R. G.: *Proc. Soc. Exper. Biol. & Med.* **65**:102, 1947. (b) Cavelti, P. A.: Studies on the Pathogenesis of Rheumatic Fever: I. Experimental Production of Autoantibodies to Heart, Skeletal Muscle and Connective Tissue, *Arch. Path.* **44**:1 (July) 1947; II. Cardiac Lesions Produced in Rats by Means of Autoantibodies to Heart and Connective Tissue, *ibid.* **44**:13 (July) 1947. More and others.<sup>12</sup> Junghans.<sup>11</sup>

6. Moore and others.<sup>5a</sup> Cavelti.<sup>5b</sup>

7. (a) Murphy, G. E., and Swift, H. F.: *J. Exper. Med.* **91**:485, 1950. (b) Aschoff, L.: *Verhandl. d. deutsch. path. Gesellsch.* **24**:13, 1929. (c) More and others.<sup>12</sup>

8. Aritz.<sup>12</sup> More and McLean.<sup>13</sup> More and others.<sup>12</sup>

9. Rich and Gregory.<sup>10c</sup> Bruun.<sup>10</sup> McKeown.<sup>11</sup> Moore and others.<sup>5a</sup>

10. (a) Miller, C. P.: *J. Exper. Med.* **40**:543, 1924. (b) Gray, F. G.: *Am. J. Path.* **25**: 1215, 1949.

11. Loewe, L., and Lenke, S. E.: *J. Exper. Med.* **71**:89, 1940.

12. Clark, E., and Kaplan, B. I.: Endocardial, Arterial and Other Mesenchymal Alterations Associated with Serum Disease in Man, *Arch. Path.* **24**:458 (Oct.) 1937.

13. More, R. H.; McMillan, G. C., and Duff, G. L.: *Am. J. Path.* **22**:703, 1946.

14. Freund, J., and McDermott, K.: *Proc. Soc. Exper. Biol. & Med.* **49**:548, 1942.

15. Kabat, E. A.; Wolf, A., and Bezer, A. E.: *J. Exper. Med.* **85**:117, 1947. Morgan, I. M.: *Ibid.* **85**:131, 1947. Cazzullo, C. L., and Ferraro, A.: Isoallergic Encephalomyelitis Produced in Guinea Pigs via Intramuscular and Intraperitoneal Injection of Antigen, *Arch. Path.* **48**:316 (Oct.) 1949.

type to the tuberculin type<sup>16</sup> when this antigen was combined with Freund's adjuvant. Accordingly, experiments were carried out to observe the effects of combining the serum antigens, horse serum and bovine gamma globulin, with Freund's adjuvant.

In view of the interest in group A beta hemolytic streptococci as etiological agents in human rheumatic fever, it was also decided to incorporate the killed and dried organisms of this group in Freund's adjuvant and inject them into rabbits (group 5). For the same reason, killed and dried streptococci were suspended in horse serum or bovine gamma globulin solutions and administered to rabbits intravenously (groups 1 and 2).

In addition to cardiac lesions, necrotizing arteritis, proliferative glomerulonephritis and bilateral renal cortical necrosis were also produced in these experiments and studied histologically.

#### MATERIALS AND METHODS

*Animals and Histological Technic.*—Albino rabbits of both sexes obtained from several dealers were employed throughout the experiments. They were fed a commercial rabbit chow<sup>17</sup> and water ad libitum, and were singly caged. They weighed 1,400 to 2,400 Gm. at the start of treatment. Control animals were left untreated and were killed at intervals throughout the

TABLE 1.—Protein Contents of the Horse Serum Used as Antigen

	Total Protein, Gm./100 Cc.	Albumin, Gm./100 Cc.	Globulin, Gm./100 Cc.
Batch 1.....	6.90	2.9	4.0
Batch 2.....	6.05	1.15	3.8

experimental periods. In all, 30 control animals were available for study. At the end of the experimental period the animals were killed by air injection or by a blow on the head and the tissues fixed in Zenker-formaldehyde solution (Helly's fluid) and embedded in paraffin. Sections were stained with the following stains: hematin, phloxine and saffron; Masson's trichrome stain; Mallory's phosphotungstic acid-hematoxylin; Weigert's elastica and fibrin stains; Glynn's modification of Gram's stain; toluidine blue, erythrosine, orange G, as required.

The heart, the kidneys, the spleen, the liver, the thymus and the adrenals of every one of the animals was studied. In many instances the other tissues were also subjected to microscopic examination. The heart was cut transversely in the plane of the atrioventricular sulcus, the upper section including 1 to 2 mm. of tissue on either side of it. The next block was a transverse section inferior to the one just mentioned, and a third block including a vertical section of the remaining portions of both ventricles was also made in most instances. This method of cutting the heart presented a large portion of the valves for examination, as well as the points at which they were attached to the fibrous ring of the atrioventricular sulcus.

*Serum Antigens.*—The horse serum employed intravenously and intracutaneously in these experiments was obtained from the Institute of Microbiology of the University of Montreal and was used after Seitz filtration and without preservative. The serum was received in two batches, and the content of protein was analyzed by Dr. Douglas Waugh, with the results shown in table 1.

The bovine gamma globulin was obtained in the dried state.<sup>18</sup> For intravenous use, it was dissolved in isotonic sodium chloride solution (1:10) and passed through a Seitz filter.

16. Dienes, L., and Schoenheit, E. W.: J. Immunol. **19**:41, 1930. Freund and McDermott.<sup>14</sup>

17. The chow used is produced by the Ralston Purina Company, Woodstock, Ont., Canada.

18. From Armour and Company, Chicago.

*Streptococci*.—A strain of beta hemolytic streptococci of Lancefield group A isolated from the throat in a case of scarlet fever was used.<sup>19a</sup> This was grown in pure culture in tryptose phosphate broth (Difco), yielding an average of 0.30 Gm. of organisms (dried weight) per liter. The bacteria were thoroughly washed in saline solution and killed by repeated changes of cold acetone as described by Cavelti.<sup>20</sup> For intravenous, intracutaneous and intramuscular injection, the dried organisms were suspended in saline solution—a suspension containing 25 mg. per cubic centimeter. The appropriate amount of suspension was drawn into the serum antigen solution in the syringe just prior to the injection.

*Freund's Adjuvant Mixtures*.—Freund's adjuvant was prepared as described by Freund and McDermott<sup>14</sup> from a strain of human tubercle bacilli (H37Rv).<sup>19b</sup>

Horse serum was obtained in the dried state and incorporated in the adjuvant in a final concentration of 0.6 Gm. (dry weight) per 5 cc. of the adjuvant mixture. The bovine gamma globulin was present in a concentration of 1 Gm. (dry weight) per 4 cc. of the mixture. The dried antigens were first suspended in saline solution. Two parts of the saline suspension were combined with one part of falba<sup>20</sup> in a mortar, and then the whole was mixed with two parts of liquid petrolatum<sup>21</sup> containing 2.5 mg. of heat-killed tubercle bacilli per cubic centimeter in a Waring blender.<sup>9</sup>

The streptococci (killed and dried) were incorporated in Freund's adjuvant in a final concentration of 5 mg. or 60 mg. per cubic centimeter. Each concentration was used for a separate group of rabbits.

*Experimental Groups*.—Group 1 (intravenous horse serum and streptococci). The injections were made with 10 ml. of horse serum per kilogram of body weight containing 6.25 mg. to 50 mg. of streptococci per dose. Two injections were given: the first on day 1 and the second on day 17 of the experiment. With the larger doses of streptococci a considerable mortality ensued, the animals dying 18 hours to 10 days after the first injection. Some animals died during the second injection, on the seventeenth day. Animals surviving the second injection were killed on the twentieth and twenty-fifth days. Accordingly, it was necessary to subdivide the group in consideration of the results. This subdivision was made according to the number of injections received by the animals and the time of survival, since there was no correlation between the lesions and the dose of streptococci. Thus—

- Group 1a. 1 injection, survival less than 24 hours (13 rabbits)
- Group 1b. 1 injection, survival 10 days (1 rabbit)
- Group 1c. 1 injection, survival 17-21 days (6 rabbits)
- Group 1d. 2 injections, survival 20-25 days (10 rabbits)

Group 2 (intravenous bovine gamma globulin and streptococci). An injection of 10 ml. per kilogram of body weight of a 10 per cent solution of bovine gamma globulin containing 6.25 to 50 mg. of streptococci was given on day 1 and another on day 10. On the seventeenth and twenty-second days survivors were killed. Subdivision of this group was made for the same reasons as in the horse serum group as follows:

- Group 2a. 1 injection, survival less than 24 hours (4 rabbits)
- Group 2b.  $\begin{cases} 1 \text{ injection, survival 14 days or till second injection (3 rabbits)} \\ 2 \text{ injections, survival 14 days or till second injection (3 rabbits)} \end{cases}$
- Group 2c. 1 injection, survival 20 days (1 rabbit)
- Group 2d. 2 injections, survival 17-22 days (14 rabbits)

19. (a) This strain was obtained through the courtesy of Dr. G. Kalz, of the clinical laboratory of the department of bacteriology and immunology of McGill University. (b) H37Rv was obtained through the courtesy of Dr. E. A. Kabat and Mr. H. Baker, of the department of bacteriology of the College of Physicians and Surgeons, Presbyterian Hospital, New York.

20. Falba<sup>®</sup> is a wool fat prepared so that it absorbs a high proportion of water. It is marketed by Pfaltz & Bauer, New York.

21. The liquid petrolatum used was that marketed as bayol,<sup>®</sup> F, produced by Stanco, New York.

Group 3 (horse serum and Freund's adjuvant—seven rabbits). Five cubic centimeters per kilogram of body weight of the adjuvant mixture was injected into the thigh muscles and adjacent subcutaneous tissue on day 1 and day 17 of the experiment. The animals were killed on the twenty-eighth and thirtieth days.

Group 4 (bovine gamma globulin and Freund's adjuvant—eight rabbits). Four cubic centimeters per kilogram of body weight of the adjuvant mixture was injected into the thigh muscles on the first and tenth days of the experiment and the animals were killed on the fifteenth and nineteenth days.

Group 5 (beta hemolytic streptococci [killed and dried] and Freund's adjuvant). Four cubic centimeters per kilogram of body weight of the adjuvant mixture was given to each of two groups: (a) 5 mg. dried streptococci per cubic centimeter of adjuvant—8 rabbits; (b) 50 mg. dried streptococci per cubic centimeter of adjuvant—8 rabbits.

Two injections were made, the first on day 1 and the second on day 12, into the thigh muscles and adjacent subcutaneous tissues; the animals being killed on the nineteenth, twenty-fifth and twenty-eighth days of the experiment.

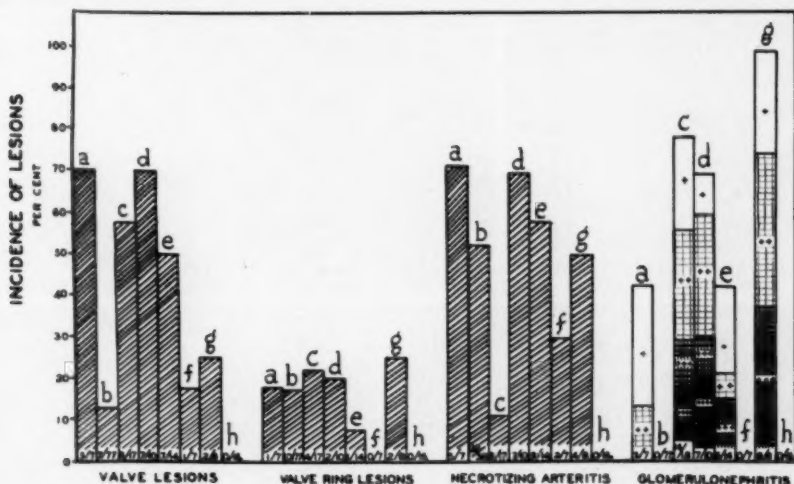


Fig. 1.—Incidence of cardiac, arterial and renal lesions in rabbits receiving two injections of foreign serum proteins in comparable doses. The various types of treatment associated with the various rates of incidence are listed below, with the ratio of the number of animals showing lesions to the number of animals treated, in each instance. I/V and I/M stand for intravenous and intramuscular injection, respectively.

#### Valve Lesions

- (a) I/V horse serum\* (5:7)
- (b) I/V horse serum† (7:77)
- (c) I/V globulin + nephrectomy‡ (8:17)
- (d) I/V horse serum + streptococci (7:10)
- (e) I/V globulin + streptococci (7:14)
- (f) I/M horse serum in Freund's adjuvant (1:7)
- (g) I/M globulin in Freund's adjuvant (2:8)
- (h) I/M streptococci in Freund's adjuvant (0:16)

#### Valve Ring Lesions

- (a) I/V horse serum\* (1:7)
- (b) I/V horse serum† (10:77)
- (c) I/V globulin + nephrectomy‡ (4:17)
- (d) I/V horse serum + streptococci (2:10)
- (e) I/V globulin + streptococci (1:14)
- (f) I/M horse serum in Freund's adjuvant (0:7)
- (g) I/M globulin in Freund's adjuvant (2:8)
- (h) I/M streptococci in Freund's adjuvant (0:16)

#### Necrotizing Arteritis

- (a) I/V horse serum\* (5:7)
- (b) I/V horse serum† (22:38)
- (c) I/V globulin + nephrectomy‡ (8:17)
- (d) I/V horse serum + streptococci (7:10)
- (e) I/V globulin + streptococci (9:14)
- (f) I/M horse serum in Freund's adjuvant (2:7)
- (g) I/M globulin in Freund's adjuvant (4:8)
- (h) I/M streptococci in Freund's adjuvant (0:16)

#### Glomerulonephritis

- (a) I/V horse serum\* (3:7)
- (b) I/V horse serum† (0:77)
- (c) I/V globulin + nephrectomy‡ (14:18)
- (d) I/V horse serum + streptococci (7:10)
- (e) I/V globulin + streptococci (6:14)
- (f) I/M horse serum in Freund's adjuvant (0:7)
- (g) I/M globulin in Freund's adjuvant (8:8)
- (h) I/M streptococci in Freund's adjuvant (0:16)

\* This was a group of 7 rabbits recently given two intravenous injections of 10 cc. per kilogram of horse serum 17 days apart.

† These results were reported by More and McLean.<sup>18</sup>

‡ The cardiac and arterial lesions were described by More, Waugh and Kobernick<sup>18</sup>; the renal lesions, by More and Waugh.<sup>28</sup>

TABLE 2.—Incidence of Lesions Produced with Horse Serum or Globulin Combined with Streptococci or with Freund's Adjuvant

Treatment	Period of Survival	Group	Ant-Group	Myocardial Lesions										Glomerulonephritis								
				Valve Lesions		Valve Ring Lesions		Arteritis		Spontaneous Rt. V.*		Near Arteries		Pericarditis		Total Incidence		Incidence of 3+ and 4+ Severity		Bilateral Cortical Necrosis		
				No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Horse serum and streptococci (intravenous)																						
1 Injection	Less than 24 hours	1a	13	0	0	0	0	0	0	2	15.4	0	0	0	0	0	0	0	0	7	53.8	
1 Injection	10 days	1b	1	1	100.0	0	0	0	0	0	0	1	100.0	0	0	0	0	0	0	1	100.0	
1 Injection	17-21 days	1c	6	2	33.3	0	0	5	83.3	0	0	3	50.0	0	0	0	0	0	0	0	0	
2 Injections	20-25 days	1d	10	7	70.0	2	20.0	7	70.0	0	0	2	20.0	2	20.0	2	20.0	7	70.0	3	30.0	
Total of 1b, 1c, 1d				17	10	58.8	2	20.0	12	70.5	2	11.7	6	35.2	2	11.7	7	41.7	3	17.6	8	50.7†
Globulin and streptococci (intravenous)																						
1 Injection	Less than 24 hours	2a	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	50.0	
1 Injection	14 days or till 24	2b	3	0	0	0	0	0	0	2	66.6	2	66.6	0	0	0	0	1	33.3	0	0	
2 Injections	30 days	2c	3	0	0	0	0	2	66.6	2	66.6	0	0	0	0	0	0	1	33.3	0	0	
2 Injections	17-22 days	2d	14	7	50.0	1	7.0	9	64.2	1	7.0	3	21.4	4	28.0	0	0	6	42.9	2	14.3	
Total of 2b, 2c, 2d				20	7	35.0	1	5.0	11	55.0	5	25.0	5	25.0	4	20.0	0	0	8	40.0	2	10.0
Horse serum in Freund's adjuvant (intramuscular)				3	7	1	16.6	0	0	2	28.6	1	16.6	0	0	0	0	0	0	0	0	0
Globulin in Freund's adjuvant (intramuscular)				4	8	2	25.0	2	25.0	4	50.0	2	25.0	0	0	0	0	0	8	100.0	3	37.5
Streptococci in Freund's adjuvant (intramuscular)				5a	8	0	0	0	0	0	0	1	12.5	0	0	0	0	0	0	0	0	0
				5b	8	0	0	0	0	0	3	37.5	0	0	0	0	0	0	0	0	0	0

\* Myocarditis of the right ventricle.

† Total of group 1a and b.

‡ Figures refer to group 2a.

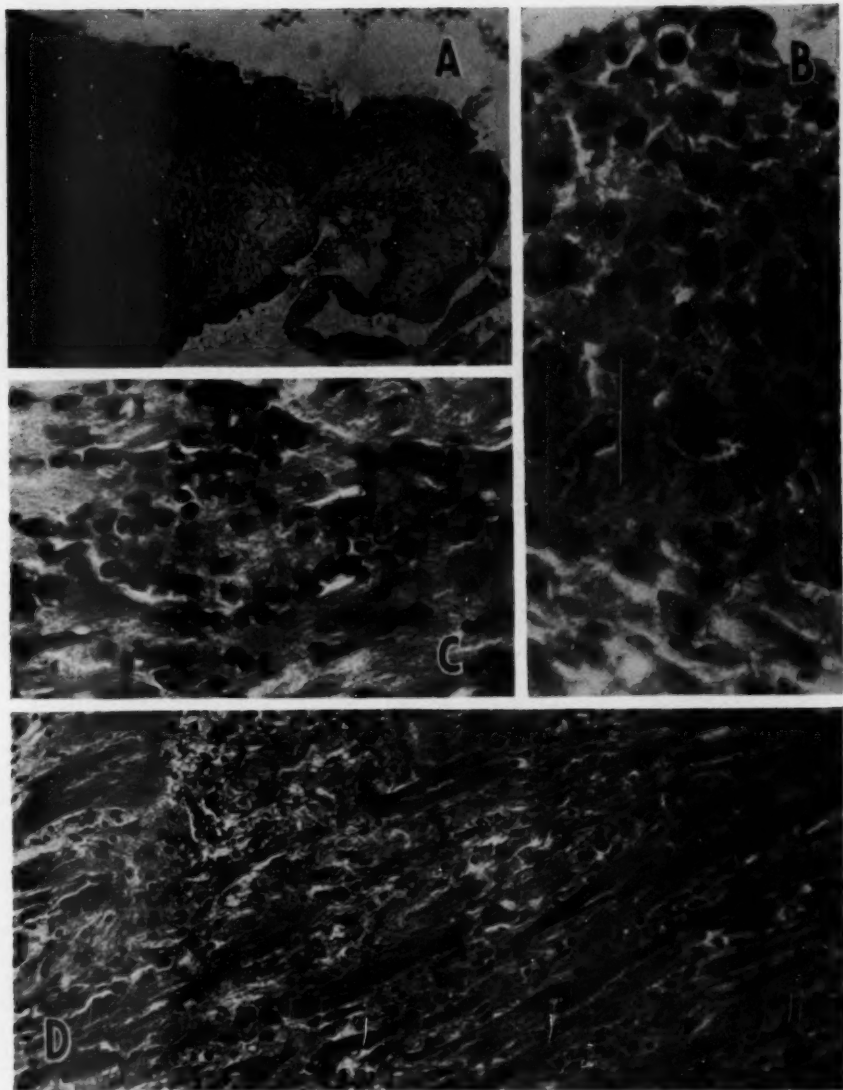


Fig. 2.—*A*, low power view of the mitral valve and valve ring of a rabbit (D-60) which had received two injections of horse serum and streptococci. It shows marked interstitial edema and infiltration and proliferation of the covering endothelium of both the superior surface and the subvalvular angle. No hyaline vegetations are seen. Hematin, phloxine and saffron;  $\times 30$ .

*B*, high power view of a portion of the valve cusp in *A*, showing damaged collagen and marked proliferation of endothelial and large mononuclear cells. Mitotic and multinucleated forms are present. Hematin, phloxine and saffron;  $\times 520$ .

*C*, spontaneous lesion of the myocardium with focal lymphocytic and larger mononuclear cell infiltration about a typically indistinct giant cell. The tissue is from an apparently normal untreated rabbit (B-38). Hematin, phloxine and saffron;  $\times 480$ .

*D*, diffuse myocarditis of the right ventricle of a rabbit (B-31) treated with horse serum and streptococci. Note diffuse destruction of myocardial fibers, replacement fibrosis, calcification of some of the remaining fibers and interstitial collections of large mononuclears. Hematin, phloxine and saffron;  $\times 250$ .

## RESULTS

*Cardiac Lesions.*—The incidence of the cardiac lesions observed in the various groups of animals is indicated in table 2. Since some confusion as to the lesions included in the term "cardiac lesions" exists in the literature, we have compared the incidence of lesions in the present experiments with that in other experiments performed in this laboratory in which rabbits received two intravenous injections of foreign serum protein alone. The comparison is indicated graphically in figure 1. It is evident from perusal of the data that there was no significant increase in the incidence of cardiac lesions when comparison was made with groups of animals receiving two intravenous injections of horse serum or of bovine gamma globulin in comparable doses. No cardiac lesions attributable to the treatment were observed in groups 5a and 5b, which received two injections of streptococci incorporated in Freund's adjuvant.

Morphologically, the lesions occurring in the hearts of the animals treated in all the experiments were the same and will be described together. The lesions of the valves and valve rings were not different in any important respects from those reported previously from this laboratory<sup>1g, h</sup> or by other authors.<sup>1a-m</sup> It was again noted that the valve lesions presented a significant absence of fibrinoid necrosis and that though some collagen damage was present (fig. 2A) the principal feature consisted of a marked proliferation of the valvular endothelium with a few infiltrating polymorphonuclear granulocytes and large mononuclear cells. No hyaline vegetations were present (fig. 2A and B). In the rings the lesions consisted of an edema of the fibrous tissue with proliferation of the fixed tissue cells, fibroblasts and histiocytes. Giant cell formation was noted, but the cells lacked the ragged cytoplasm of typical Aschoff cells.

The myocardial lesions observed in the animals may be divided into three types. The first type is one which cannot be attributed to treatment and resembles the spontaneous lesions seen in untreated rabbits (fig. 2C). These were found in 10 of 30 normal hearts examined from control material available, and were similar to the lesions reported by Miller.<sup>10a</sup> They consisted of focal accumulations of lymphocytes and a few larger mononuclear cells about small foci of degenerated muscle fibers, sometimes containing a giant cell with indistinct nuclei. Some of these lesions were quite extensive and might be located in the myocardium of any chamber of the heart.

## EXPLANATION OF FIGURE 3

Fig. 3.—A, small myocardial artery of a rabbit (D-64), treated with two injections of globulin and streptococci, showing a relatively anuclear media, a smooth muscle cell in mitosis and a marked adventitial infiltrate of large mononuclear cells and some multinucleated forms. A few lymphocytes are also evident. Hematin, phloxine and saffron;  $\times 460$ .

B, paravascular mononuclear infiltration about a small myocardial artery. The indefinite changes in the media of this artery are accompanied by more florid lesions in other arteries of the heart. The rabbit (B-17) was treated with globulin and streptococci. Hematin, phloxine and saffron;  $\times 460$ .

C, acute arteritis of a small myocardial artery of a rabbit (D-34) treated with globulin and streptococci, showing fibrinoid necrosis of the adventitia with paravascular accumulation of large mononuclear cells. Degenerative changes are also present in the media, and the endothelium is swollen. Hematin, phloxine and saffron;  $\times 475$ .

D, spontaneous lesion of a small myocardial artery of the left ventricle of a normal untreated rabbit (D-62), showing concentric intimal thickening by collagenous fibers with an occasional foam cell. The media is irregular, and increased collagen is also present in the adventitia, where there is an increase in large mononuclear cells. Weigert's elastica stain and hematin, phloxine and saffron;  $\times 118$ .

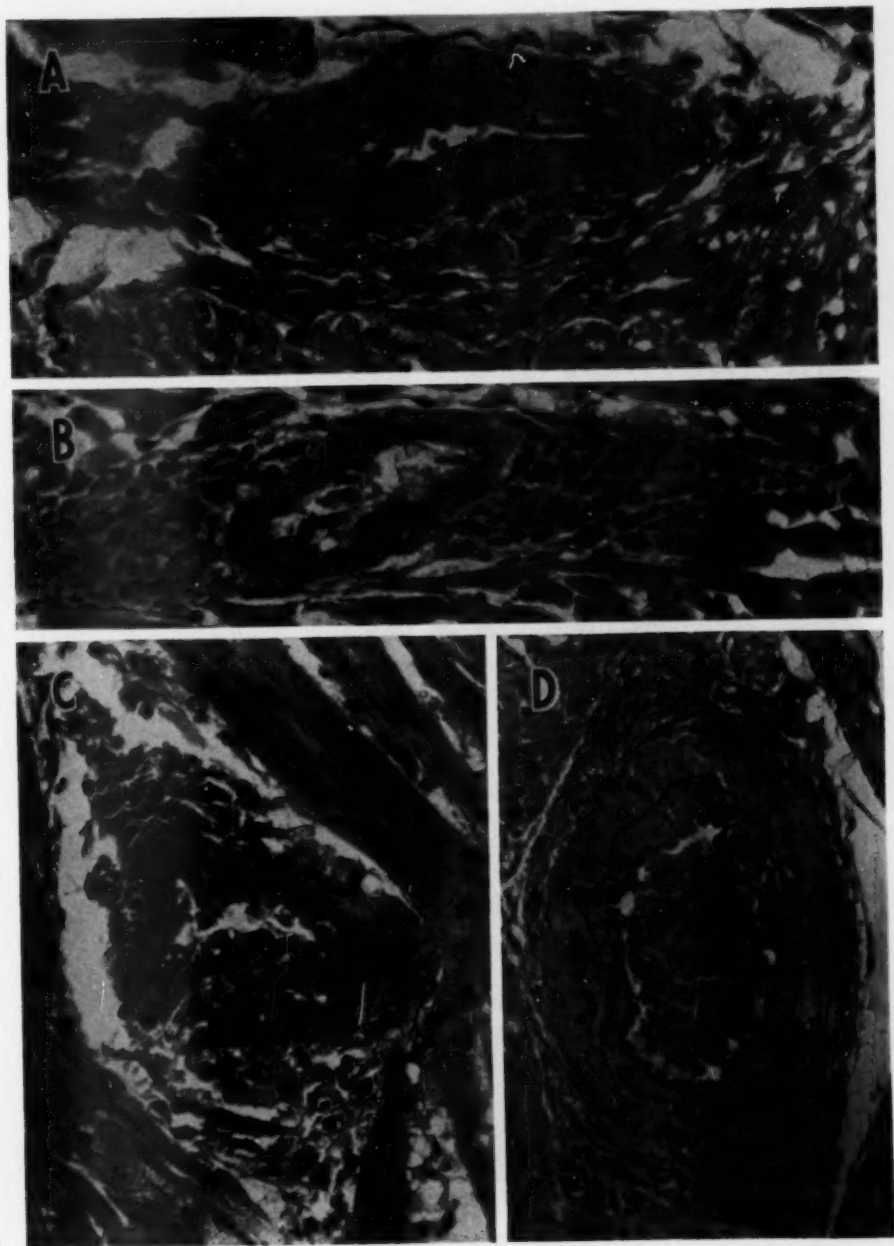


Figure 3

(See legends on opposite page)

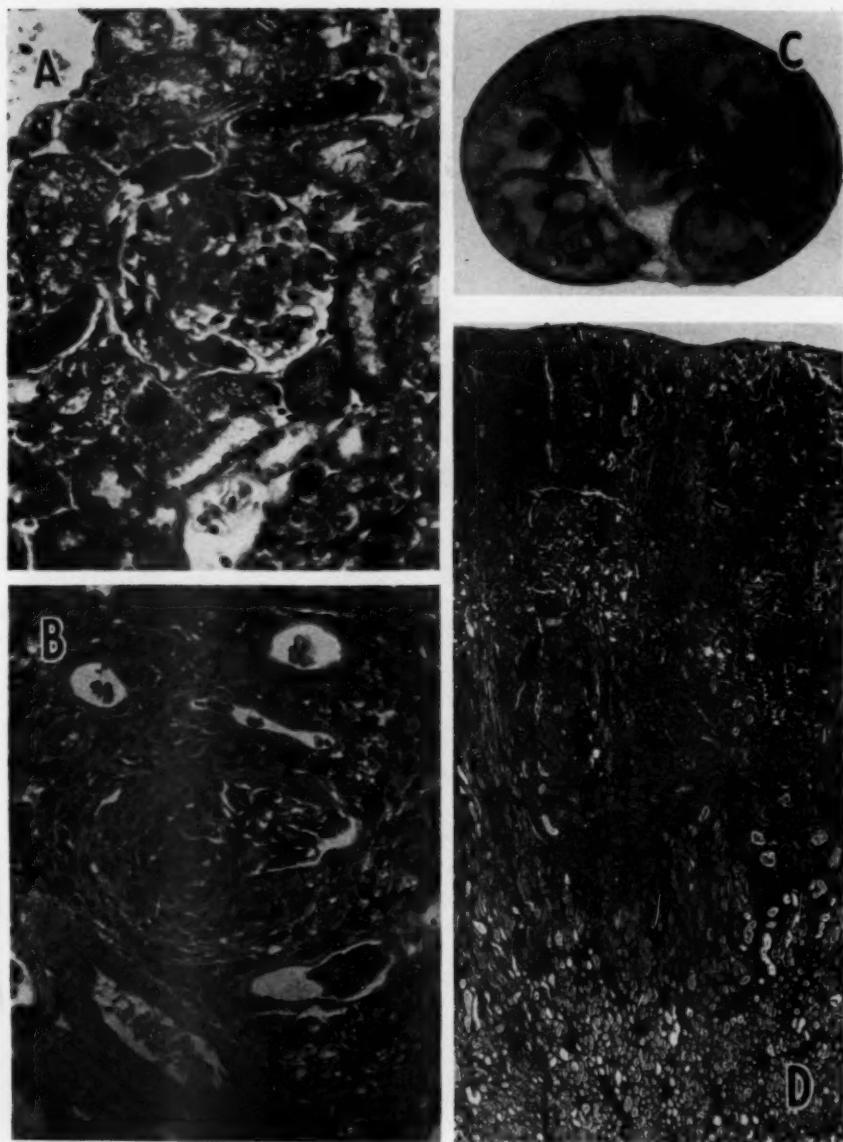


Figure 4

(See legends on opposite page)

The second type of lesions is one which may be attributed to the treatment and is designated as diffuse myocarditis of the right ventricle (fig. 2D). The incidence of this lesion is indicated in table 2. Morphologically, it differed from the spontaneous lesions in extensiveness, often occupying large areas of the ventricle and consisted of a destruction of muscle fibers with a diffuse infiltration of large mononuclear cells. Giant cells were often prominent in the lesions, and calcification of the degenerated muscle fibers was occasionally present. This lesion is similar to that previously described.<sup>22</sup>

The third type of myocardial lesion is that which occurs near arteries (fig. 3A, B and C). This lesion consists of focal accumulations of large and small mononuclear cells with occasional giant cells in the adventitia of small myocardial arteries. Some of the arteries on which these lesions center are the seat of active necrotizing arteritis, and in these instances fibrinoid necrosis of the collagen is sometimes evident mingling with the inflammatory exudate (fig. 2C). Other arteries show evidence of prior inflammation (fig. 3A). In some animals the interstitial paravascular infiltrations are associated with arteries which are not clearly the seat of active or old degenerative changes (fig. 2B). However, in all such instances arteritis was present in other locations in the same heart, and in view of the segmental nature of the arteritis it is entirely probable that these localized accumulations of cells are related to necrotizing arteritis of segments of the arteries just out of the plane of section from the inflammatory infiltrations.

*Glomerulonephritis.*—The incidence of glomerulonephritis in the various experiments is indicated in the table 2 and in figure 1. It can be seen that glomerulonephritis occurred in the animals treated with horse serum and streptococci, globulin and streptococci or horse serum alone, and in the groups receiving globulin in Freund's adjuvant. The nephritis which appeared in the animals treated with horse serum and streptococci differs from that reported in the literature as due to horse serum in that lesions of the 3+ and 4+ varieties were seen (fig. 4A and B).

Morphologically, the lesions in the kidney involved almost every glomerulus and were characterized by proliferation of the endothelial and epithelial cells of the

22. Apitz.<sup>1f</sup> More and McLean.<sup>1h</sup> Ehrich and others.<sup>1k</sup>

#### EXPLANATION OF FIGURE 4

Fig. 4.—A, experimental glomerulonephritis of the kidney of a rabbit (D-45) treated with two injections of horse serum and streptococci. The condition, graded 3+, consists of endothelial and epithelial proliferation in the tuft, with obliteration of many capillaries, and casts in the tubules and hyaline droplets in the tubular epithelium of the proximal convoluted tubules at the lower left. Hematin, phloxine and saffron;  $\times 300$ .

B, experimental glomerulonephritis, grade 4+, of a rabbit (D-32) treated with two injections of horse serum and streptococci. Note obliteration of a portion of the tuft, a well developed crescent, focal lymphocytic infiltration, atrophy of the tubules and casts. Hematin, phloxine and saffron;  $\times 300$ .

C, cut surface of a kidney of a rabbit (B-33) dead 29 hours after an intravenous injection of horse serum and streptococci. It shows the distribution of pale infarction surrounded by a zone of hyperemia. The cortex is almost entirely necrotic, and many areas in the medulla are also involved. The specimen is seen slightly larger than natural size, formaldehyde fixed.

D, section of a kidney from a rabbit (B-31) 19 days after an intravenous injection of horse serum and streptococci, showing extensive coagulative necrosis of the cortex with preservation of the medulla. No emboli were found in the renal vessels. Hematin, phloxine and saffron;  $\times 30$ .

tuft, a slight increase in the number of polymorphonuclear granulocytes in the tuft, narrowing and obliteration of capillaries (fig. 4A), crescent formation, and thickening and splitting of the basement membranes (fig. 4B). Protein exudate was present in the capsular spaces and hyaline casts in the tubules (fig. 4A and B). Hyaline droplets were observed in the cells of the proximal convoluted tubules (fig. 4A), and some of the cells of Henle's loops showed hydropic vacuolation. The lesions were graded 1, 2, 3 or 4+ according to criteria already described.<sup>2a</sup>

*Bilateral Renal Cortical Necrosis.*—Bilateral cortical necrosis of the kidneys was observed in 53.8 per cent of the animals in group 1a, the one animal in group 1b, and in 50 per cent of the animals in group 2a (table 2). The condition consisted of a grossly visible pale mottling of the external surfaces of both kidneys accompanied with slight enlargement and softness of the organs. On cut section, small areas of the whole thickness of the cortex and discrete areas of the medulla were pale gray to bright yellow. In lesions observed later a zone of hyperemia appeared about them (fig. 4C). In two instances in group 1a only the gross lesions were seen, but in all the others there was evidence of coagulative necrosis of the renal parenchyma in the areas corresponding to the pallor seen in the gross specimens (fig. 4D). Only in the lesion present for 10 days was a marginal zone of polymorphonuclear granulocyte infiltration observed about the necrotic areas. There was no evidence of embolism or thrombosis of renal vessels of any size.

In each animal in which the renal lesion was observed, most of the lung capillaries and venules were occluded by masses of amorphous material containing gram-positive cocci. In the animal that survived 10 days the lungs were free of any lesion, and one can only assume that the pulmonary emboli had been dissipated in the interval while the coagulative necrosis of the kidney persisted. Emboli were not present in any other organs.

*Necrotizing Arteritis.*—The incidence of necrotizing arteritis is detailed in table 2 and figure 1. Arteritis appeared in all animals receiving two injections of horse serum or of bovine gamma globulin whether these were combined with streptococci or with Freund's adjuvant (groups 1d, 2d, 3 and 4) and in animals receiving one injection of horse serum or globulin with streptococci that lived 17 to 22 days after the first injection (groups 1c and 2c). (It is of interest that one animal in group 1c died on the seventeenth day of a ruptured mesenteric artery, the rupture having occurred through a necrotic area in the wall.) No arterial lesions were present in the animals treated with streptococci in Freund's adjuvant (groups 5a and 5b). The highest incidence of arteritis occurred in group 1c, closely followed by that in group 1d, but in no group was the incidence of arteritis significantly increased over that occurring in the groups of animals treated in previous experiments.

The lesions of the arteries were similar morphologically to those previously reported.<sup>2a</sup> However, whereas other authors have emphasized the necrosis of the media, it was apparent from the study of earlier material and from the present experiments that in the acute phase the earliest necrosis was seen in the collagen of the adventitia, extending to the fine collagen fibers between the muscle cells of the media (fig. 5A). The necrosis was accompanied by an infiltration of large mono-

23. More and others.<sup>18</sup> More and McLean.<sup>1b</sup> Rich and Gregory.<sup>2a</sup> Fox and Jones.<sup>2b</sup> Knepper and Waaler.<sup>2c</sup> Hopps and Wissler.<sup>1a</sup>

nuclear cells and a few polymorphonuclear granulocytes (fig. 1). The muscle cells of the media were not greatly affected in the large arteries of the heart, showing swelling of the cytoplasm and nucleus which was reversible (fig. 5 C), and the muscle was restored to a relatively normal state when the acute stage subsided, with an increase in thickness of the collagen marking the site of the fibrinoid necrosis between the muscle cells (fig. 5 C and D). It was rather excep-

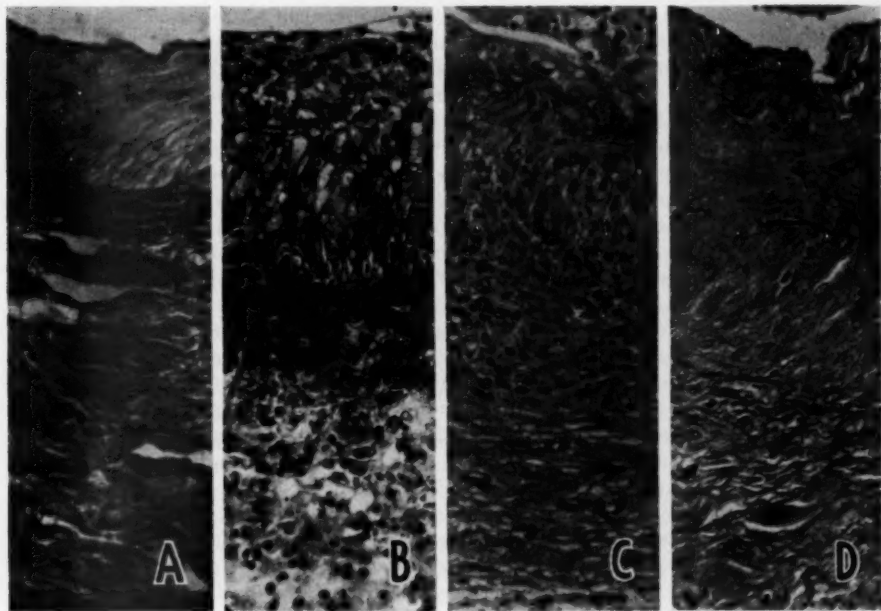


Fig. 5.—A, normal large coronary artery from an untreated rabbit, showing the arrangement of the media with the wavy internal elastica immediately beneath the endothelium. The external elastic fibers occupy almost the whole of the adventitia. Myocardial muscle is seen below. Weigert's elastica stain and hematin, phloxine-saffron;  $\times 250$ .

B, acute necrotizing arteritis of a large coronary artery showing fibrinoid necrosis of the collagen of the adventitia amid the external elastic fibers. Necrotic collagen fibers can also be seen extending between the muscle cells of the media, which show intracellular edema, swelling and relatively little pyknosis. The internal elastica is straight, attenuated and interrupted. There is prominent subendothelial edema with an infiltrate of large mononuclear cells and a few polymorphonuclear granulocytes, similar in character to the adventitial exudate. The rabbit (D-60) was treated with two injections of horse serum and streptococci. Weigert's elastica stain and hematin, phloxine-saffron;  $\times 250$ .

C, subacute necrotizing arteritis of a large coronary artery from a rabbit (D-32) treated with two injections of horse serum and streptococci, showing medial muscle cells restored to a more normal state, thin internal elastica, fewer inflammatory cells in the intima but conspicuous fibrinoid material in this location. The adventitial exudate is concentrated in the region of the external elastica, and there is a frank increase in the periadventitial collagen. Weigert's elastica stain and hematin, phloxine-saffron;  $\times 250$ .

D, chronic (healed) arteritis of a large coronary artery from a rabbit (D-62) treated with one injection of horse serum and streptococci, showing the advanced intimal collagenous thickening with elastification of the subendothelial layers, interruption of the internal elastica, separation of the external elastic fibers and increased collagen in the adventitia but relatively little increase in the thickness or the collagen content of the media. Weigert's elastica stain and hematin, phloxine-saffron;  $\times 250$ .

tional to see actual necrosis of the muscle cells of the media as the most prominent feature. The external elastic fibers were mingled with the fibrinoid necrotic material of the inner adventitia (fig. 5 C), while the internal elastica appeared attenuated and interrupted in some areas (fig. 5 B and C). The intima showed sub-endothelial edema and infiltration of mononuclear cells, a few polymorphonuclear granulocytes and fibrinoid material (fig. 5 B and C). Subsequently, the intima manifested a fibrous intimal thickening (fig. 5 D). The exudate accompanying the acute necrosis of the adventitial collagen eventually disappeared, and the adventitia appeared as if thickened by an increase in collagen (fig. 5 C and D).

The location of the arterial lesions is of interest since it appeared that with horse serum the lesions occurred predominantly in the larger arteries of the heart and other organs (fig. 5), while with bovine gamma globulin the lesions tended to involve the smaller intramyocardial branches of the coronary arteries (fig. 3 A, B and C). An exception to this statement is the fact that the arterial lesions—the few present—in group 3 (horse serum and Freund's adjuvant) involved the small arteries. In the experiments in which bovine globulin was used in conjunction with streptococci or in Freund's adjuvant, the arterial lesions tended to be more acute than in the hearts of animals treated with intravenous globulin in saline solution alone.

In the present study a review of control material and of the hearts of the treated animals reveals that there is a variety of arterial lesion which must be regarded as spontaneous since it was seen in 10 of the 30 control hearts examined. These lesions consisted of a concentric intimal fibrous thickening, sometimes containing a few foam cells, and a fibrous thickening of the adventitia containing a few lymphocytes or large mononuclears (fig. 3 D). The arteries most often affected by this spontaneous lesion were located in the posterior papillary muscle and the adjacent inner layers of the myocardium of the left ventricle. When arteritis due to treatment was present in other small myocardial arteries, it usually involved these arteries as well. When these arteries were affected in the manner described without any other arteries being clearly involved, their involvement was not included in the results as arteritis resulting from treatment.

#### COMMENT

The results of these experiments indicate that incorporating either horse serum or bovine gamma globulin in Freund's adjuvant or combining the antigens with intravenous streptococci does not alter the morphological aspect of the lesions of the valves, valve rings or arteries of the heart as compared with the lesions produced by intravenous administration of the same quantity, by weight, of the foreign serums alone.

We have sought a myocardial lesion that is qualitatively different from the lesions heretofore reported as resulting from the injection of foreign serum proteins for the reason that if the criterion for the ultimate diagnosis of rheumatic fever is the Aschoff body in the myocardium, one must find lesions in animals identical with one or more of the typical stages of the human disease in order to state that the experimental and the human disease are similar. To admit lesions having only a "similar reaction pattern" to comparison with the human disease is to start with atypical lesions on which no decision as to identity can be made. With this standard in mind it is apparent that all the lesions hitherto reported in animals

treated with foreign serum proteins do not demonstrate an identity between the experimental and human lesions. They are, on the other hand, similar to the lesions described in human serum sickness<sup>12</sup> or sulfonamide allergy in man.<sup>13</sup> While this attitude has been regarded as purist,<sup>24</sup> it is apparent from the work of Murphy and Swift<sup>25</sup> that the purists have not sought a closer morphological approximation to the Aschoff body in vain. Having been privileged to view the material of the last-named investigators, we feel convinced that a focal interstitial myocarditis embodying the characteristics of an Aschoff body, including fibrinoid necrosis of the collagen and mononuclear cell infiltration with giant cells which are very similar to the Aschoff cells of human rheumatic fever, has been produced. These lesions occurred in the absence of appreciable arteritis.

The similarity between the necrotizing arteritis of animals produced by sensitization to foreign serum proteins and the lesions of human periarteritis nodosa has been repeatedly pointed out.<sup>26</sup> Though the morphological structure of the lesions in periarteritis nodosa varies a great deal from case to case and description of the lesions from one report to another,<sup>27</sup> it seems pertinent to draw attention to the similarity between the lesions observed in our animals—particularly the early tendency to involve the adventitia and the collagen fibrils of the media, sparing the muscle cells from necrosis until later in the evolution of the lesions—and the observation by Duff<sup>28</sup> that a similar situation obtains in the early lesions of human cases of periarteritis nodosa. It is also well to point out that the predominantly mononuclear reaction is similar to that observed in the lesions of periarteritis nodosa in man.<sup>29</sup>

It is also of interest to note that, although the morphologic appearance of individual lesions is the same in animals treated in different ways, they may be located in arteries of different sizes. Thus, in animals given two massive intravenous injections of horse serum alone or combined with streptococci, the arteritis occurred in the larger arteries of the heart. However, when horse serum was combined with Freund's adjuvant and administered intramuscularly, the lesions tended to occur in the smaller myocardial arteries, a location similar to that observed when bovine gamma globulin was injected intravenously<sup>18</sup> or intramuscularly in combination with Freund's adjuvant. Whether the altered location of the lesions induced by horse serum is related to the intramuscular route of injection or to the combination with Freund's adjuvant is a matter to be settled by further investigation. The fact that the lesions of the arteries produced by horse serum administered in this manner were similar to those induced by bovine gamma globulin may be of importance in the understanding of the genesis of the lesions. One further fact remaining to be explained is that the lesions occurring as a result of the intramuscular injection of bovine globulin in Freund's adjuvant were much more acute than those obtained by the intravenous injection of the globulin alone.<sup>18</sup>

The glomerular lesions produced in these experiments were morphologically similar in every way to those present in animals treated with two intravenous

24. Aegerter, E., and Long, J. H.: *Am. J. M. Sc.* **218**:324, 1949.

25. Murphy, G. E., and Swift, H. F.: *J. Exper. Med.* **89**:687, 1949; **91**:485, 1950.

26. Rich and Gregory.<sup>2a</sup> More and McLean.<sup>1b</sup>

27. Zeek, P. M.; Smith, C. C., and Weeter, J. C.: *Am. J. Path.* **24**:889, 1948.

28. Duff, G. L.: *Canad. M. A. J.* **58**:317, 1948.

29. More, R. H.: *Am. J. Path.* **26**:702, 1950.

injections of bovine gamma globulin<sup>30</sup> or with horse serum<sup>30</sup> after the animals had been subjected to unilateral nephrectomy. Study of these lesions again indicated the striking similarity between them and the lesions of human acute and subacute glomerulonephritis. However, one of the most significant findings in these experiments was the florid glomerulonephritis (+++ and +++) which appeared in animals treated with intravenous horse serum and streptococci. Such lesions have hitherto been known to result from administration of horse serum only when the animals were unilaterally nephrectomized before the serum was administered.<sup>30</sup> Horse serum incorporated in Freund's adjuvant did not cause glomerulonephritis. The glomerulonephritis that resulted from injection of bovine gamma globulin in Freund's adjuvant occurred in a higher incidence than heretofore observed in nephrectomized<sup>30</sup> or non-nephrectomized animals.<sup>1d</sup> However, when streptococci were added to the globulin administered intravenously there was no similar increased incidence of the lesions. Thus it appears that when horse serum is used as antigen, the addition of streptococci increases the incidence and severity of glomerulonephritis, an effect similar to that of nephrectomy. Freund's adjuvant does not appear to exert a similar effect. When, however, bovine gamma globulin is employed as the antigen, Freund's adjuvant appears to increase the incidence of the lesions, while streptococci do not. Whether these results are completely fortuitous or whether they have some significance in the genesis of experimental glomerulonephritis cannot at present be stated.

It was of interest that a lesion appeared in animals treated with intravenously injected horse serum or globulin and streptococci which had not been seen in animals treated with the serum antigens alone. This was bilateral cortical necrosis of the kidneys. It is pertinent to this observation to state that 24 hours prior to the intravenous injection all the animals, including those that did not and those that did manifest this lesion, had received an intradermal injection of killed streptococci suspended in the serum antigen and in saline solution, to determine the skin reactivity to the mixtures and to the dead bacterial bodies themselves. In 24 hours there was a tiny 2 to 3 mm.-sized area of necrosis at the site of the intradermal injection with an area of redness 2 to 3 mm. wide around it. No hemorrhage was evident following the intravenous injection. It may be a vague possibility that the reaction in the kidney was a manifestation of the Schwartzman phenomenon as postulated in other experiments by Black-Schaffer, Hiebert and Kerby.<sup>31</sup> With the hemorrhage obscured by the necrosis, however, there is nothing in the present experiments to confirm this possibility. On the other hand, it was a consistent finding in the animals which succumbed within 24 hours after the intravenous injection that capillaries and arterioles of the lungs were filled with embolic material containing many stainable gram-positive cocci, but none were demonstrated in the renal vessels. It is thus possible to attribute the shocklike state which followed the injection in these instances to extensive embolism of the pulmonary circulation; the shock may have caused a relative ischemia of the kidney or spasm of the arterioles, with consequent necrosis of the renal parenchyma analogous to the

30. McLean, C. R.: Unpublished data. Masugi and Sato.<sup>2d</sup>

31. Black-Schaffer, B.; Hiebert, T. G., and Kerby, G. P.: Experimental Study of Purpuric Meningococcemia in Relation to Schwartzman Phenomenon, with Discussion of Meningococcic Purpura, Waterhouse-Friderichsen Syndrome and Bilateral Renal Cortical Necrosis, *Arch. Path.* **43**:28 (Jan.) 1947.

mode of reaction observed by Trueta and associates<sup>32</sup> and others.<sup>33</sup> No emboli were visible in any of the renal vessels, and when necrosis of the arterioles was observed, it was in areas of necrotic parenchyma. Conglutination thrombi were evident in some of these vessels, but there was no evidence that these had preceded the necrosis of the parenchyma.

This lesion is, in our experience, peculiar to the animals that received dead streptococci intravenously in addition to the serum antigens. In view of the specific finding of massive bacterial embolism in the lungs, there appears to be no reason to assume a possible hypersensitive basis for this experimental variety of bilateral renal cortical necrosis.

Thus we did not succeed in producing cardiac lesions resembling Aschoff bodies in the hearts of rabbits by any of the technics described in these experiments. The lesions of the heart and arteries were similar to those reported previously as resulting from massive injections of foreign serum proteins. In the same way, the experimental glomerulonephritis was similar, morphologically, to that previously reported. However, it was apparent that the modifications employed were variously capable of enhancing the incidence and the severity of the lesions of both the heart and the kidneys, though discrepancies of efficacy were evident between individual technics. A new lesion, that of bilateral cortical necrosis of the kidneys, was observed to occur in animals that died soon after the intravenous injection of serum antigens in which streptococci were suspended. These results appeared to be of sufficient interest and importance to warrant their inclusion in the literature.

#### SUMMARY

Groups of rabbits were given two intravenous injections of horse serum or bovine gamma globulin combined with killed and dried group A beta hemolytic streptococci. Other groups of rabbits were given two intramuscular injections of horse serum or bovine gamma globulin or killed and dried group A streptococci in Freund's adjuvant.

The experimental technics employed were not successful in producing lesions of the myocardium similar to those seen in human rheumatic fever. It is also pointed out that the lesions of the hearts of rabbits produced by foreign serum proteins in these and in other published experiments were not identical with the lesions of human rheumatic fever and that a closer identity is required before any valid analogy can be made between them.

The groups of animals receiving the serum proteins manifested necrotizing arteritis and glomerulonephritis as well as cardiac valvulitis and myocarditis. In a few rabbits receiving injections of serum proteins and streptococci intravenously, bilateral renal cortical necrosis was seen. No lesions of any organs appeared in the animals receiving streptococci in Freund's adjuvant.

Morphologically, the lesions of the arteries were similar to those previously reported in the literature, bearing a close resemblance to the lesions seen in many cases of human periarteritis nodosa. The glomerulonephritis was regarded as

32. Trueta, J.; Barclay, A. E.; Daniel, P. M.; Franklin, K. J., and Prichard, M. M. L.: *Studies of the Renal Circulation*, Springfield, Ill., Charles C Thomas, Publisher, 1947.

33. Duff, G. L., and More, R. H.: *Am. J. Sc.* **201**:428, 1950. Byrom, F. B.: *J. Path. & Bact.* **45**:1, 1937.

acceptably similar to human proliferative glomerulonephritis in the acute and subacute stages. The bilateral renal cortical necrosis was observed only in animals succumbing to the injection of serum protein and streptococci, and the lesions were similar to those of human patients with this disease. It was accompanied by massive occlusion of lung capillaries by emboli containing bacterial masses, but there was no evidence of embolism in the vessels of the kidneys. The lesions were therefore attributed to spasm of the renal arterioles.

The only significant differences in lesions between these and previously reported experiments were observed in the case of the proliferative glomerulonephritis, lesions of the reported severity never having been previously observed to occur from the administration of horse serum without prior unilateral nephrectomy; also in the high incidence of the glomerulonephritis in the group receiving bovine gamma globulin in Freund's adjuvant.

## EFFECT OF TWEEN 80<sup>®</sup> ON THE SERUM LIPIDS AND THE TISSUES OF CHOLESTEROL-FED RABBITS

TORRENCE P. B. PAYNE, M.D., Ph.D.

AND

G. LYMAN DUFF, M.D., Ph.D.

MONTREAL, CANADA

RECENTLY Kellner and co-workers<sup>1</sup> studied the effects of certain commercial detergents on the development of experimental cholesterol atherosclerosis in the rabbit with very interesting results. In their first experiment<sup>1a</sup> they fed polysorbate 80 U. S. P. (tween 80<sup>®</sup>)<sup>1a</sup> soirethytan (20) monooleate to rabbits in a cholesterol-rich diet and obtained blood cholesterol levels two to three times as high as those obtained by cholesterol feeding alone. Moreover, the tween 80<sup>®</sup>-fed rabbits exhibited an earlier and slightly more severe degree of atherosclerosis than did the controls. In a subsequent experiment<sup>1b</sup> these investigators reported that when tween 80<sup>®</sup> or triton A-20,<sup>®</sup> another synthetic detergent, was injected intravenously into cholesterol-fed rabbits, there occurred a rise in serum phospholipids which was parallel with the increase in serum cholesterol, and in these circumstances the development of atherosclerosis was inhibited.

Because of our recent demonstration<sup>2</sup> that the relation of serum cholesterol to the other serum lipids and to the serum proteins is even more important than the absolute level of cholesterol itself in the genesis of experimental cholesterol atherosclerosis, we have had occasion to study the effects of tween 80,<sup>®</sup> given orally and intravenously, on the serum lipids and the tissues of cholesterol-fed rabbits. Our results appear to be of sufficient importance to be placed on record.

### MATERIALS AND METHODS

Nineteen male adult albino rabbits, weighing between 2.0 and 3.0 Kg., were used. All the animals were fed a cholesterol-rich diet prepared as follows: A solution of cholesterol (B. D. H.) in ether was mixed with the food (purina<sup>®</sup> laboratory chow), and the ether was allowed to evaporate completely before the food was placed in the animal's cage. The proportion of cholesterol to food was 1 Gm. to 100 Gm. The daily dose of cholesterol was 1 Gm. (in 100 Gm. food). The rest of the diet was made up of noncholesterolized food as required. When tween 80<sup>®</sup> was administered orally, the daily dose was 10 cc., and it was mixed with the

Dr. Payne is Research Fellow of the American Heart Association.

From the Department of Pathology, Pathological Institute, McGill University.

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1. (a) Kellner, A.; Correll, J. W., and Ladd, A. T.: *Proc. Soc. Exper. Biol. & Med.* **67**:25, 1948. (b) Ladd, A. T.; Kellner, A., and Correll, J. W.: *Federation Proc.* **8**:360, 1949.

1a. The more descriptive term "soirethytan (20) monooleate" for the polyoxyethylene derivative sorbitan monooleate has been accepted by the Council on Pharmacy and Chemistry of the American Medical Association (*J. A. M. A.* **145**:646 [March 3] 1951) as its generic name for this product.

2. Duff, G. L., and Payne, T. P. B.: *J. Exper. Med.* **92**:299, 1950.

cholesterolized food.<sup>3</sup> For intravenous injection, a 20 per cent solution of the detergent, made up with isotonic solution of sodium chloride, was used. It was injected twice daily, the second injection being made approximately nine hours after the first one. For the first two weeks of the experiment 10 cc. of the 20 per cent solution of the detergent was used for each injection. Thereafter, the dose was increased to 15 cc., injected twice a day.

Serum lipid determinations were made at intervals of approximately two weeks. Blood was drawn in the nonfasting state from the central artery of the ear and the serum separated. In the case of the rabbits receiving tween 80<sup>®</sup> intravenously, the blood was drawn between six and 12 hours after the last injection of the detergent, since it is at this time that the maximum lipid levels are attained.<sup>4</sup> Half of the serum was used immediately for the determination of the absolute levels of the lipid constituents. Total fatty acids were determined by the method of Stoddard and Drury as modified by Man and Gildea<sup>5</sup>; lipid phosphorus, by the method of Youngburg as modified by Hawk, Oser and Summerson<sup>6</sup>; free and total cholesterol, by the method of Schoenheimer and Sperry as modified by Sperry.<sup>7</sup> Neutral fat was calculated as

*Serum Lipids in Normal Rabbits Fed Cholesterol and Treated with Tween 80<sup>®</sup>*

Average Serum Lipid Values Expressed in Proportion to a Serum Total Cholesterol of 100 Mg./100 Cc.										
No.	Experimental Type *	Duration of Cholesterol Feeding, Days	Total Dose of Choles- terol, Gm.	Average Total Serum Choles- terol, Mg./ 100 Cc.	Average Serum Lipid Values Expressed in Proportion to a Serum Total Cholesterol of 100 Mg./100 Cc.			Grade of Atherosclerosis (0-4)		
					Fatty Acids of Neutral Fat, mEq.	Lipid Phos- phorus Mg./ 100 Cc.	Cholesterol, Mg./100 Cc.			
							Total		Free	Ester
F48	C	65	57	986	1.1	1.8	100	42	58	1
F49	C	..	..	990	1.4	1.8	100	50	50	4
F50	C	..	..	795	3.1	1.9	100	56	44	3
F51	C	..	..	418	2.2	1.8	100	36	64	1
F31	TF	65	57	1130	1.3	1.6	100	41	59	3
F37	TF	..	..	1317	4.4	2.5	100	55	45	2
F42	TF	..	..	1403	1.8	1.8	100	51	49	2
F52	TF	..	..	1545	1.9	1.5	100	44	56	2
F47	TF	..	..	1073	1.5	1.9	100	46	54	1
137	TI	65	57	1087	1.6	3.5	100	63	37	0
143	TI	..	..	1383	1.8	3.6	100	61	39	1 (minimal)
136	TI	..	..	1223	1.6	3.0	100	62	38	0
144	TI	..	..	1157	1.2	4.7	100	73	27	0
141	TI	40	38	1375	2.2	3.8	100	66	34	0
138	TI	45	42	1545	1.7	3.8	100	48	52	0
145	TI	47	35	990	1.7	3.5	100	60	40	0
139	TI	53	39	1465	0.7	3.2	100	52	48	0
140	TI	52	46	1065	4.1	4.9	100	68	32	0
135	TI	61	41	1477	3.0	3.1	100	55	45	0

<sup>a</sup> C = control; TF = fed tween 80<sup>®</sup>; TI = tween 80<sup>®</sup> injected intravenously.

described by Peters and Man.<sup>8</sup> The other half of the serum was rapidly frozen and then dried *in vacuo* and extracted overnight with chloroform in the cold, according to the method of Forbes and associates.<sup>9</sup> The chloroform was then evaporated *in vacuo* at a temperature below 40 C., and determinations of total fatty acids, lipid phosphorus and free and total cholesterol were made on the residue as above. The values obtained in the latter way will be referred to as values for the "readily extractable" lipid fractions, representing, according to Forbes and associates,<sup>9</sup> the lipid fractions either not bound or only loosely bound to protein.

3. Tween 80<sup>®</sup> is manufactured by the Atlas Powder Company, Wilmington, Del.

4. Kellner, A.; Correll, J. W., and Ladd, A. T.: *Federation Proc.* **8**:359, 1949.

5. Man, E. B., and Gildea, E. F.: *J. Biol. Chem.* **99**:43, 1932.

6. Hawk, P. B.; Oser, B. L., and Summerson, W. H.: *Practical Physiological Chemistry*, ed. 12, Philadelphia, The Blakiston Company, 1947.

7. Sperry, W. M.: *Am. J. Clin. Path. Tech. Supp.* **2**:91, 1938.

8. Peters, J. P., and Man, E. B.: *J. Clin. Investigation* **22**:707, 1943.

9. Forbes, J. C.; Dillard, G. H. L.; Porter, W. B., and Petterson, O.: *Proc. Soc. Exper. Biol. & Med.* **68**:240, 1948.

Elsewhere<sup>10</sup> we have presented suggestive evidence in support of the validity of this method. At the end of the cholesterol-feeding period, the rabbits were killed by air embolism and autopsies made. The tissues were fixed in formaldehyde-saline solution and Zenker-formaldehyde solution (Helly's fluid), and selected blocks were prepared for histological study. The heart and aorta were removed en bloc. The aorta was carefully opened and the degree of atherosclerosis recorded by means of schematic drawings and graded on an arbitrary scale of 0 to 4.

#### RESULTS

The serum lipid findings are summarized in the accompanying table. It should be stated that in all the rabbits there occurred an elevation of all the serum lipid constituents. However, in order to show the increase of serum cholesterol in relation to the elevation of the other serum lipids, in the table the average serum lipid values are expressed in proportion to the average serum total cholesterol, which has been arbitrarily set at 100 mg. per 100 cc. In this way the ratios of serum neutral fat and serum lipid phosphorus to serum total cholesterol in different animals can be readily compared. The animals fall into three groups: four control animals fed cholesterol only; five rabbits fed cholesterol and tween 80®; four rabbits fed cholesterol and given intravenous injections of tween 80®. All of these animals completed a 65 day course of cholesterol feeding, during which each rabbit received 57 Gm. of cholesterol. In addition, six animals which received tween 80® intravenously but died before completing the course of cholesterol feeding are listed at the foot of the table. The values for the "readily extractable" lipid fractions are not included in the table, since it was found that with the lipemic serums of the tween 80®-treated animals, these values approximated the absolute lipid values just as they did with the serums of those rabbits which were fed cholesterol alone.

It will be seen that the rabbits which were fed cholesterol and tween 80® showed a somewhat higher level of serum cholesterol than the controls, but the other lipids were also correspondingly elevated, so that the ratios of total cholesterol to lipid phosphorus and total cholesterol to neutral fat remained the same as in the controls. Moreover, these animals did not have a greater degree of atherosclerosis than the controls. The rabbits receiving the detergent intravenously also showed a somewhat higher level of serum cholesterol than the controls and, as was expected, the proportionate increase of lipid phosphorus was much greater than in the controls. However, the fatty acids of neutral fat were not markedly elevated. In none of these rabbits was more than the slightest degree of aortic atherosclerosis observed, although even those animals listed at the foot of the table would be expected to show a moderate degree of atherosclerosis if fed cholesterol alone.

In the animals given intravenous injections of tween 80® the rise in serum cholesterol was due much more to a rise in free cholesterol than to an increase in ester cholesterol, while in the rabbits fed cholesterol and tween 80® and in those fed cholesterol alone (as is already known) the greater increment of cholesterol tended to be in the ester fraction.

In the tissues of the rabbits receiving tween 80® intravenously there were very interesting findings, which, so far as we are aware, have not been previously reported. In every case the spleen was tremendously enlarged, weighing as much

10. Payne, T. P. B., and Duff, G. L.: *Proc. Soc. Exper. Biol. & Med.* **73**:332, 1950. Duff and Payne.<sup>2</sup>

as 20 times the normal weight. Microscopically (fig. 1 *A*), the splenic tissue was composed almost entirely of closely packed mononuclear cells which had a "foamy" cytoplasm. The sinusoids were filled with these foam cells, which were also present

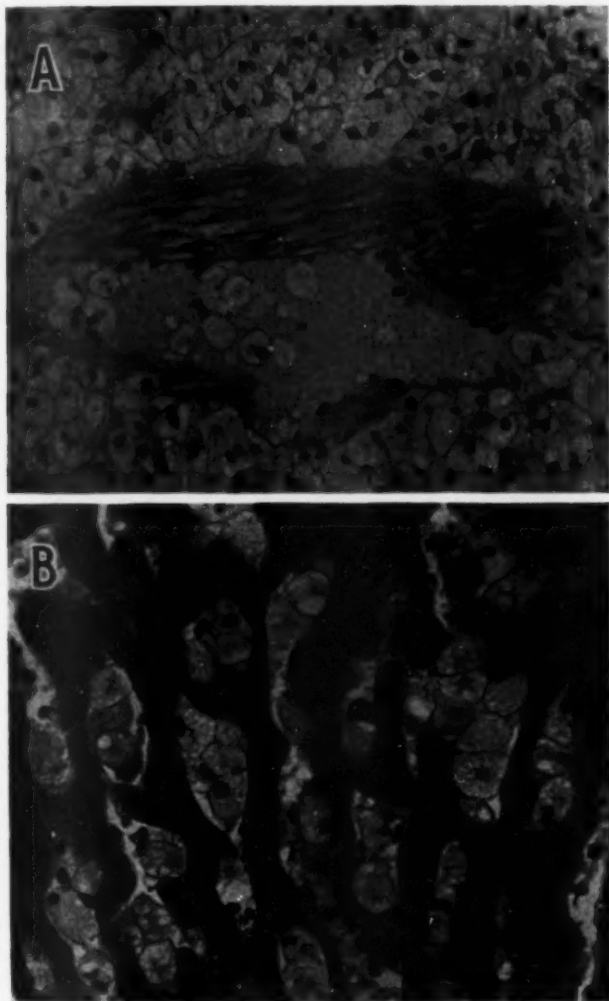


Fig. 1.—*A*, spleen showing abundance of foam cells in splenic tissue. Note several foam cells in the lumen of a small vein. *B*, liver showing sinusoids filled with foam cells.

in the blood vessels. Numerous foam cells were present also in the liver sinusoids and, to a lesser extent, in the sinuses of lymph nodes. In some cases the focal accumulations of these cells in the hepatic sinusoids caused atrophy of the surround-

ing liver parenchyma (fig. 1 *B*). In the lungs alveolar capillaries were filled with foam cells, and these cells were also present in the blood vessels (fig. 2 *A*). In the kidney, foam cells were present in the glomerular capillaries, and the cells of

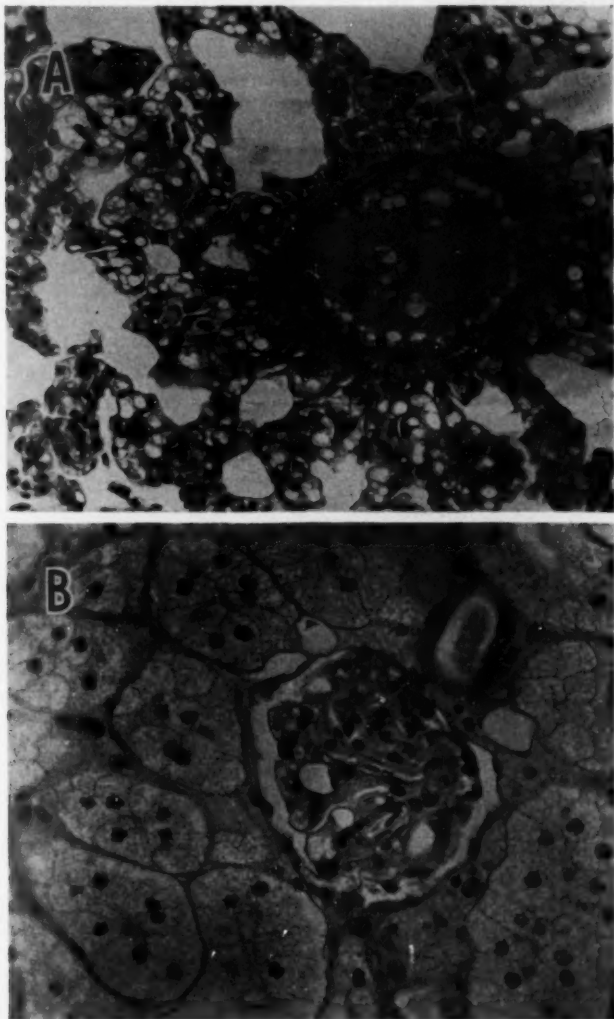


Fig. 2.—*A*, lung. The alveolar capillaries are filled with foam cells. Foam cells are also present in the lumen of a small vein. *B*, kidney showing foam cells in the glomerular capillaries. Note the marked swelling and fine vacuolation of the cells of the proximal convoluted tubules.

the convoluted tubules were enlarged and their cytoplasm was vacuolated (fig. 2 *B*). Vacuolation of the liver parenchymal cells was not marked. All the foam cells, as

well as the cells of the convoluted tubules of the kidney, contained sudanophilic and doubly refractile material with a positive Liebermann reaction for cholesterol.

The lesion in the kidneys described above and the tremendous accumulation of foam cells were not seen in the rabbits which were fed cholesterol alone, or in those fed cholesterol and tween 80.\*

Microscopic examination of the blood vessels showed minimal deposits of lipids and accumulations of foam cells in the subendothelial tissue of some of the aortas and in small arteries of the spleens and lungs of the cholesterol-fed rabbits which received injections of tween 80.\* These lesions did not differ in any essential respect from the early lesions observed in the arteries of rabbits fed cholesterol alone. Plugging of the vasa vasorum with foam cells was not demonstrated.

#### COMMENT

The finding that in the lipemic serums of the tween 80\*-treated rabbits the greater proportion of the serum lipids was "readily extractable" (i. e., unbound or only loosely bound to the serum proteins), irrespective of whether the development of atherosclerosis was accelerated or inhibited, is in keeping with our previous demonstration<sup>2</sup> that, although looseness of linkage of the serum lipids and the serum proteins may be conducive to lipid deposition in the arterial walls, other conditions must also be satisfied before deposition occurs.

So far as the oral use of tween 80\* is concerned, it should be pointed out that in the rabbits fed cholesterol and tween 80\* the serum cholesterol levels were not quite as high as those reported by Kellner and associates.<sup>1</sup> This may well be due to the fact that oil was not added to the diet in our experiment, while it was in theirs. Serum cholesterol was the only lipid constituent determined in their study, so that no correlation between the ratio of cholesterol to the other serum lipids and the slightly more severe degree of atherosclerosis reported in their tween 80\*-treated rabbits was made. At any rate, although only a small number of animals were used, our results provide suggestive evidence that, other things being equal, a greater degree of hypercholesteremia will not result in the production of earlier and more extensive arterial lesions so long as there is a proportionate elevation of the other serum lipids.

In the rabbits receiving tween 80\* intravenously, the results confirmed the finding of Kellner and his associates that the phospholipids rise almost parallel with the serum cholesterol. The fatty acids of neutral fat, however, were not significantly elevated in our study, although Ahrens and Kunkel,<sup>11</sup> who examined serum from the cholesterol-fed rabbits receiving tween 80\* intravenously in Kellner's original experiment, reported high values for the serum neutral fat. This apparent discrepancy may be explained by the fact that their analysis was made by the gasometric procedure of Van Slyke, the neutral fat being determined by weight, while our analysis was made by the titration method which determines the combining power of the fatty acids. Since it is highly probable that both methods measure also the injected circulating tween 80,\* which is composed of the salt of oleic acid with a long chain carbon compound (polymer), it would be expected that a value determined by weight would be higher than one obtained by titrating only the oleic acid portion of the molecule. It appears likely, therefore, that considerable amounts

11. Ahrens, E. H., and Kunkel, H. G.: *J. Exper. Med.* **90**:409, 1949.

of tween 80® remain in the blood stream for long periods. Thus it would seem that the inhibitory effect of intravenous tween 80® on the development of experimental cholesterol atherosclerosis cannot with surety be attributed merely to the elevated phospholipids; tween 80® may well have an inhibitory effect of itself and independent of the changes produced in the serum lipids.

The observation that in the animals receiving intravenous injections of tween 80® the rise in serum cholesterol was due much more to a rise in free cholesterol than to an increase in ester cholesterol has already been made.<sup>11</sup> Its significance, however, is not clear. Hemolysis was not noted as a result of the intravenous injection of tween 80®, although some degree of hemolysis might be expected when a detergent is injected into the blood stream. Moreover, elevated free cholesterol may be found in hemolyzed blood.<sup>12</sup>

The tissue changes which result from the intravenous injection of tween 80® in cholesterol-fed rabbits are of great interest mainly for two reasons. In the first place, they constitute evidence that intravenous tween 80® in the doses used here is not by any means a bland substance, but causes definite damage to the kidneys and other vital organs. Accordingly, it should not be used clinically as a possible therapeutic weapon against human atherosclerosis without further study. Secondly, the finding that in the rabbits which received injections of tween 80® the reticulo-endothelial system was loaded with doubly refractile lipid material in spite of the fact that the aorta contained only minimal deposits of lipid argues against the validity of the theory suggested by Leary<sup>13</sup> and more recently adopted by Dauber and Katz<sup>14</sup> and Gordon<sup>15</sup> to explain the genesis of both human and experimental cholesterol atherosclerosis. According to this theory, cholesterol is introduced into the subendothelial tissue by cholesterol ester-containing lipophages that penetrate the intact endothelial lining from the blood stream or enter the arterial wall via the vasa vasorum. These cholesterol-carrying lipophages are supposed to be derived from desquamated lipid-filled reticuloendothelial cells of the liver, the spleen and the lungs. In the rabbits fed cholesterol and given intravenous injections of tween 80®, although there existed a great abundance of these lipophages not only in these organs but also in the circulating blood stream, the arterial lesions observed were only minimal, and no plugging of vasa vasorum with foam cells could be demonstrated. On the other hand, in the control animals, in which the accumulation of foam cells in the reticuloendothelial system was by no means so impressive, severe atherosclerosis developed.

#### SUMMARY

A study of the serum lipids and the tissues of rabbits fed cholesterol and treated by oral or intravenous administration of the polysorbate 80 U. S. P. (tween 80®) was made.

The study of the serum lipids provided suggestive supporting evidence for our previous demonstration that the relation of serum cholesterol to the other serum

12. Landé, K. E., and Sperry, W. M.: Human Atherosclerosis in Relation to Cholesterol Content of Blood Serum, *Arch. Path.* **22**:301 (Sept.) 1936.

13. Leary, T.: Genesis of Atherosclerosis, *Arch. Path.* **32**:507 (Oct.) 1941.

14. Dauber, D. V., and Katz, L. N.: Experimental Atherosclerosis in Chick, *Arch. Path.* **36**:473 (Nov.) 1943.

15. Gordon, I.: Mechanism of Lipophage Deposition in Atherosclerosis, *Arch. Path.* **44**:247 (Sept.) 1947.

lipids and to the serum proteins is even more important than the absolute level of cholesterol itself in the genesis of experimental cholesterol atherosclerosis. However, the inhibition of atherosclerosis in rabbits fed cholesterol and given tween 80<sup>®</sup> intravenously cannot with surety be attributed merely to the elevated phospholipids. It appears likely that the injected detergent remains in the blood stream for long periods of time, so that it may well have an inhibitory effect, of itself and independent of the serum lipid changes, on the development of atherosclerosis.

Examination of the tissues revealed that in cholesterol-fed rabbits intravenous tween 80<sup>®</sup> in the doses used here produces tremendous foam cell accumulation in the reticuloendothelial system and marked lipid infiltration of the renal tubular epithelium. These findings are of interest for two reasons: In the first place, they suggest that the clinical use of intravenous injections of tween 80<sup>®</sup> in human atherosclerosis may not be without dangerous side effects; secondly, the finding of tremendous accumulations of lipid-filled foam cells in the reticuloendothelial system in spite of the fact that the aorta contains only minimal deposits of lipid argues against the validity of the lipophagy theory of the genesis of atherosclerosis as propounded by Leary,<sup>13</sup> Dauber and Katz<sup>14</sup> and Gordon.<sup>15</sup>

## POSSIBLE PATHOGENIC MECHANISMS RESPONSIBLE FOR HUMAN PERIARTERITIS NODOSA

As Suggested by the Occurrence of Two Instances of This Disease in Association with Glomerulonephritis

G. F. KIPKIE, M.D.  
KINGSTON, ONT., CANADA  
AND  
D. S. JOHNSON, M.D.  
DURHAM, N. C.

**K**NOWLEDGE of the cause and pathogenesis of periarteritis is of particular importance with the apparent increase in the number of cases which are appearing in the literature. Recently, within two weeks, two cases of glomerulonephritis were observed in Duke Hospital, both with interesting arteritis. These cases are thought worthy of a report since they suggest one or more possible pathogenic mechanisms which may be involved in the production of periarteritis nodosa.

### REPORT OF CASES

**CASE 1.**—A 7 year old white boy became ill 12 weeks before death with a severe cold and sore throat. This was treated with one of the sulfonamide compounds. Three weeks later a diagnosis of acute nephritis was made by the attending physician subsequent to urinalysis and observation of periorbital edema. The child was edematous when admitted to the hospital; the blood pressure was elevated to 140/120, and the heart was thought to be enlarged. Laboratory studies confirmed the diagnosis and showed elevation of nonprotein nitrogen, creatinine and cholesterol. The patient was treated with penicillin without effect. During hospitalization he had a continuous fever, oliguria throughout the entire course and evidence of increasing nonprotein nitrogen retention. He died in respiratory distress nine weeks after the original diagnosis of nephritis.

At autopsy there was fluid in the body cavities, the lungs were edematous and there was so much edema of the vocal cords that the larynx was almost completely closed. The kidneys were swollen and pale, with numerous 1-4 mm. areas of hemorrhage on the surfaces. The heart was hypertrophied and dilated. A firm, pale gray mural thrombus was present in the right auricular appendage. The microscopic examination revealed subacute glomerulonephritis with extensive proliferation of the endothelium of the glomerular capillaries and numerous epithelial crescents. In the interlobular arteries of the kidney, the intrahepatic branches of the hepatic artery, the arteries and arterioles of the gastrointestinal tract (fig. 1A) and those of the periaortic connective tissue and the testes (fig. 1B) a necrotizing arteritis was found. Polymorphonuclear granulocytes, large mononuclear macrophages, occasionally plasma cells and rarely eosinophils were found surrounding the vessels so that panarteritis or periarteritis was produced. The vessels were involved in a segmental fashion. No aneurysms were present. Occasionally the lumen of a vessel would be almost obliterated by endothelial swelling or by fibrinoid necrotic debris, but no thrombi were seen.

**Anatomic Diagnoses.**—Subacute diffuse glomerulonephritis; left-sided hypertrophy and dilatation of the heart; pulmonary edema; mural thrombus (right auricular appendage); periarteritis nodosa (generalized).

From the Department of Pathology, Duke University School of Medicine.

CASE 2.—An 11 year old white boy was admitted to Duke Hospital two weeks before death, complaining of "swelling of the stomach and puffiness around the eyes" of five days' duration.

The boy had been a blue baby at birth and was known to have congenital heart disease, the exact nature of which was undetermined. He had bouts of exertional dyspnea and tired easily. At the age of 6 years an electrocardiogram revealed marked right axis deviation.

Five weeks before death the patient had a throat infection with high fever and cough. He was treated with a sulfonamide drug, although he had shown a reaction to sulfonamides, consisting of weakness and dizziness, eight months before death. Two and one-half weeks after the infection of the upper respiratory tract, the patient's abdomen enlarged, and there were periorbital edema, anorexia, a notable gain in weight and increased shortness of breath. He became oliguric, and the urine was dark.

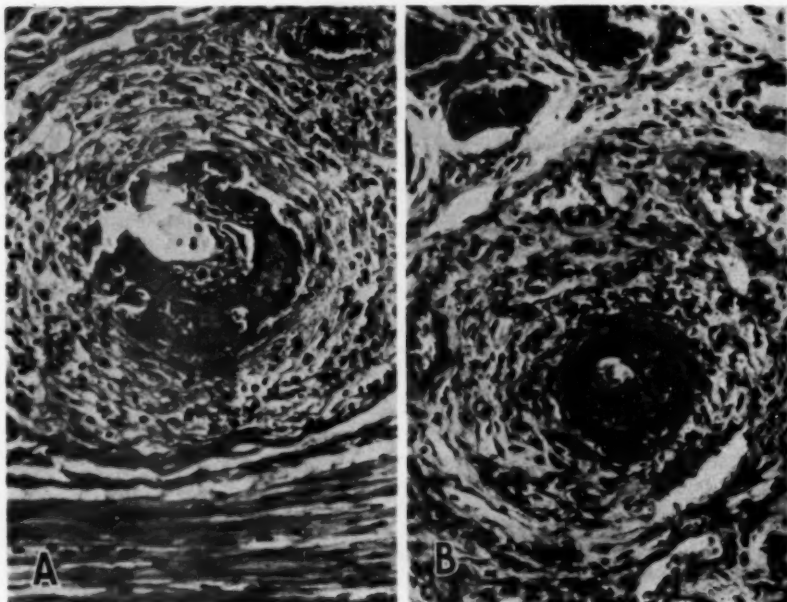


Fig. 1 (case 1).—*A*, periarteritis nodosa in the submucosa of the intestine; hematoxylin and eosin stain;  $\times 400$ . *B*, periarteritis nodosa in a testis showing necrosis of the vessel wall and inflammatory cell infiltration; hematoxylin and eosin stain;  $\times 585$ .

His blood pressure was elevated to 146/118, ascites was present, and the heart status appeared as before. Laboratory studies showed elevated blood nonprotein nitrogen and normal serum cholesterol. The patient was digitalized and given penicillin in adequate dosage. The elevation of blood pressure persisted during hospitalization as did that of nonprotein nitrogen. He died 35 days after the throat infection.

At autopsy, fluid was present in the body cavities. The heart malformation was of the Eisenmenger type with a high interventricular septal defect, hypertrophy of the right ventricle and overriding of the septal defect by the aorta. The mitral valve had a narrow band of connective tissue measuring 1-2 mm. in width and 1 mm. in thickness situated on the auricular side. This ran in a circumferential manner about the valve. The kidneys were slightly swollen, pale gray, with petechiae scattered over the surface. The glomeruli were swollen.

exhibiting proliferation of endothelium and epithelium covering the glomerular tufts. Moderate infiltrations of polymorphonuclear granulocytes were present. Rarely, epithelial casts were seen. Hyaline and red cell casts were noted in the tubules. The medium-sized and small pulmonary arteries exhibited necrotizing arteritis (fig. 2 *A* and *B*). Numerous large mononuclear cells, plasma cells and lymphocytes and small numbers of polymorphonuclear granulocytes and eosinophils were present in the vessel wall and periadventitial tissues. The involved vessels were situated near bronchi but were not bronchial arteries. The arterial changes varied, ranging from swelling of the endothelium with proliferation of endothelial cells to an intense inflammatory reaction with fibrinoid necrosis. Some of the lesions were healed, and in these vessels the lumens were obliterated by dense fibrous connective tissue arranged in concentric rings. Rarely, multinucleated giant cells were found in healing and healed areas. No other arteries in the body were involved in this process.

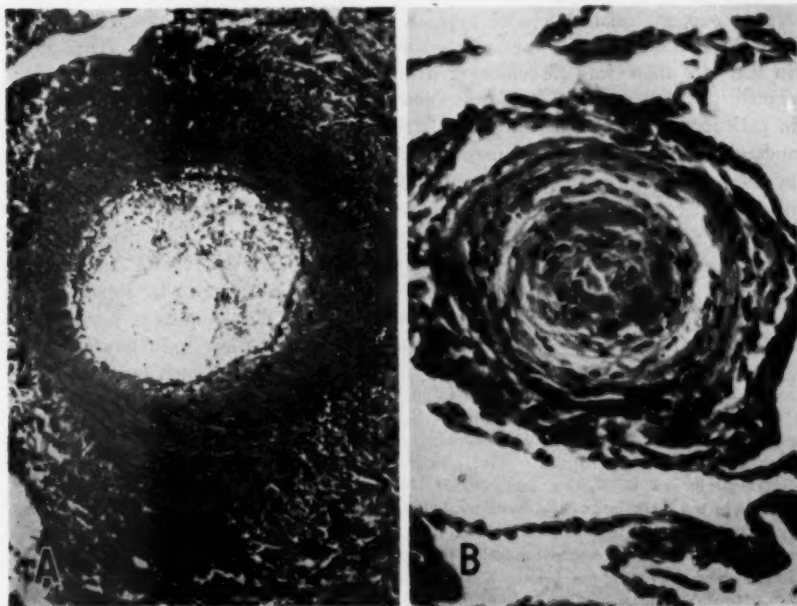


Fig. 2 (case 2).—*A*, acute periarteritis in a lung showing segmental involvement of the vessel; hematoxylin and eosin stain;  $\times 300$ . *B*, pulmonary artery, filled with hyaline material, the entire wall of which is infiltrated with inflammatory cells; hematoxylin and eosin stain;  $\times 400$ .

rings. Rarely, multinucleated giant cells were found in healing and healed areas. No other arteries in the body were involved in this process.

*Anatomic Diagnoses.*—Acute diffuse glomerulonephritis; cardiac malformation (Eisenmenger's complex); hypertrophy of the heart; periarteritis nodosa confined to pulmonary arteries.

#### COMMENT

The operation of one or several mechanisms in the production of periarteritis nodosa is suggested in these two cases. The possibilities would appear to be as follows: (1) The arteritis was due to a pathogenic process which was the same as that responsible for the glomerulonephritis; (2) it was the result of the hypertension, or (3) it was an allergic reaction to a sulfonamide compound.

Both patients had glomerulonephritis, acute in one, subacute in the other. The extensive experimental studies of glomerulonephritis made by numerous investigators<sup>1</sup> lend support to the allergic or sensitization concept of the causation of the disease. If this concept be true, the arteritic lesions seen in these patients may be the result of the reaction of fibrous connective tissue and ground substance of arteries to the same unknown sensitizing agent which caused the nephritis. However, the distribution of the lesions in the two patients is hard to understand unless one postulates in each case different organs on which the sensitizing agent is capable of acting.

The distribution of lesions in these two patients is interesting and suggests certain etiologic relationships to hypertension. In case 1 the lesions were in the systemic circulation, while in case 2 they were confined to the pulmonary circulation. In this case there was Eisenmenger malformation of the heart. It is difficult to explain this distribution. It is well known that lesions such as we see here occur in patients with hypertension, particularly in the malignant phase of the disease<sup>2</sup> and with the development of uremia. Both patients had hypertension, systemic in case 1, systemic and pulmonary in case 2. If hypertension alone were the chief mechanism responsible for the lesions, we would naturally expect to find lesions in the systemic as well as in the pulmonary circulation. To our knowledge, only one case of periarteritis confined exclusively to the pulmonary arteries has as yet been reported.<sup>3</sup> This concerned a young child with a high interventricular septal defect. Some of the factors increasing the susceptibility to the development of periarteritis limited to the pulmonary circulation, as in case 2, may be inferred from the work of Courmand, Baldwin and Himmelstein,<sup>4</sup> Dexter<sup>5</sup> and Welch and Kinney.<sup>6</sup> Stroke

1. (a) Cavelti, P. A., and Cavelti, E. S.: Studies on the Pathogenesis of Glomerulonephritis: I. The Production of Autoantibodies to Kidney in Experimental Animals, *Arch. Path.* **39**:148-152 (March) 1945; (b) II. Production of Glomerulonephritis in Rats by Means of Autoantibodies to Kidney, *ibid.* **40**:158-162 (Sept.) 1945; III. Clinical and Pathologic Aspects of the Experimental Glomerulonephritis Produced in Rats by Means of Autoantibodies to Kidney, *ibid.* **40**:163-172 (Sept.) 1945. (c) Longcope, W. T.: The Production of Experimental Nephritis by Repeated Protein Intoxication, *J. Exper. Med.* **18**:678-703, 1913; (d) Some Observations on the Course and Outcome of Hemorrhagic Nephritis, *Internat. Clin.* **1**:1-16, 1938. (e) Masugi, M.: Über die experimentelle Glomerulonephritis durch das spezifische Antinierenserum: Ein Beitrag zur Pathogenese der diffusen Glomerulonephritis, *Beitr. z. path. Anat. u. z. allg. Path.* **92**:429-466, 1934. (f) More, R. H., and Waugh, D.: Diffuse Glomerulonephritis Produced in Rabbits by Massive Injections of Bovine Serum Gamma Globulin, *J. Exper. Med.* **89**:541-554, 1949.

2. Wilson, C., and Byrom, F. B.: Renal Changes in Malignant Hypertension: Experimental Evidence, *Lancet* **1**:136-139, 1939.

3. Old, J. W., and Russell, W. O.: Congenital Heart Disease with Necrotizing Arteritis (Periarteritis Nodosa) Limited to the Pulmonary Arteries: Report of a Case with Necropsy, abstracted, *Am. J. Path.* **23**:903-904, 1947.

4. Courmand, A.; Baldwin, J. S., and Himmelstein, A.: Cardiac Catheterization in Congenital Heart Disease: A Clinical and Physiological Study in Infants and Children, New York, The Commonwealth Fund, 1949.

5. Dexter, L.: Mechanisms of Human Hypertension, *Am. J. Med.* **4**:279-284, 1948.

6. Welch, K. J., and Kinney, T. D.: The Effect of Patent Ductus Arteriosus and of Intraauricular and Interventricular Septal Defects on the Development of Pulmonary Vascular Lesions, *Am. J. Path.* **24**:729-761, 1948.

volume and mean pulmonary arterial pressure are the two factors determining the expended energy of the right side of the heart. In case 2 there was right-sided hypertrophy of the heart, indicating that at least one and perhaps both these factors were increased. Which factor was primarily involved cannot be stated definitely. Some patients with interventricular septal defects have increased pulmonary artery pressure, and Bing<sup>7</sup> stated that in Eisenmenger's malformation of the heart the right ventricle and pulmonary artery carry an abnormally high pressure. On the other hand, Welch and Kinney expressed the belief that a sharp increase in pulmonary blood flow is the common factor in the production of pulmonary vascular lesions. We were unable to find the lesions described by these authors as indicative of increased pulmonary blood flow. However, the periarteritic lesions in the lungs were so extensive that we cannot make a definite statement regarding the possibility of other pulmonary arterial lesions in case 2. Thus the mechanism of hypertension alone does not seem adequate to explain the arterial lesions found in these two patients.

The third mechanism which might be involved in the production of the arteritis is that of sulfonamide allergy. That lesions resembling those in our patients are found in patients exhibiting sensitivity to these drugs is well known and has been confirmed experimentally by the work of Rich<sup>8</sup> and Rich and Gregory.<sup>9</sup> Both the patients under discussion were treated with sulfonamides: the patient in case 1, 12 weeks before death, and the patient in case 2, eight months before death and again five weeks before death. The latter exhibited sensitivity to the drug the first time this treatment was given. Particularly in his case might sulfonamide allergy be thought of as the mechanism of the production of the arteritis. While we cannot deny that this is a possibility, we were unable to find the other lesions supposedly characteristic of sulfonamide allergy in man as reported by More, McMillan and Duff<sup>10</sup> and French and Weller.<sup>11</sup> Nor are we able to explain the distribution of the arterial lesions in these patients on the basis of sulfonamide allergy alone.

It is clear from the above discussion that it is impossible to ascribe the causation of the periarteritis specifically to any of the mechanisms discussed. In all probability the hypersensitivity was more important and probably was acting along with some unknown factor. However, the distribution of the lesions is not adequately explained on any basis.

7. Bing, R. J.; Vandam, L. D., and Gray, F. D., Jr.: Physiological Studies in Congenital Heart Disease: III. Results Obtained in 5 Cases of Eisenmenger's Complex, *Bull. Johns Hopkins Hosp.* **80**:323-347, 1947.

8. Rich, A. R.: The Role of Hypersensitivity in Periarteritis Nodosa as Indicated by 7 Cases Developing During Serum Sickness and Sulfonamide Therapy, *Bull. Johns Hopkins Hosp.* **71**:123-140, 1942; Additional Evidence of the Role of Hypersensitivity in the Etiology of Periarteritis Nodosa: Another Case Associated with a Sulfonamide Reaction, *ibid.* **71**:375-379, 1942.

9. Rich, A. R., and Gregory, J. E.: Experimental Demonstration that Periarteritis Nodosa Is a Manifestation of Hypersensitivity, *Bull. Johns Hopkins Hosp.* **72**:65-88, 1943.

10. More, R. H.; McMillan, G. C., and Duff, G. L.: The Pathology of Sulfonamide Allergy in Man, *Am. J. Path.* **22**:703-735, 1946.

11. French, A. J., and Weller, C. V.: Interstitial Myocarditis Following Clinical and Experimental Use of Sulfonamide Drugs, *Am. J. Path.* **18**:109-121, 1942.

## SUMMARY

Two cases of periarteritis nodosa occurring in patients with glomerulonephritis are described. Some of the mechanisms which may have been involved in the production of the periarteritis are discussed: (1) a pathogenic process that was responsible for the arteritis as well as for the glomerulonephritis; (2) hypertension; (3) an allergic reaction to a sulfonamide compound. The data presented do not permit one to assign the periarteritis nodosa occurring in these cases specifically to the operation of any one of the suggested mechanisms.

Case 2 is the second on record in which periarteritic lesions occurred exclusively in the lungs.

## FUCHSINOPHILIA IN THE ADRENAL CORTEX

A Critical Examination of the Broster-Vines Technic

ANNE D. ZINSSER, M.D.

AND

HANS H. ZINSSER, M.D.

PASADENA, CALIF.

THAT THE acidophilic staining properties of certain cells of the adrenal cortex are associated with sexual function has been reported for many animal species (amphibians,<sup>1</sup> fowls, ground squirrels, rabbits,<sup>2</sup> opossums,<sup>3</sup> cats<sup>4</sup> and mice,<sup>5</sup> as well as for primates<sup>6</sup> and man).

In man, considerable attention has been directed to fuchsinophilia in the fetal adrenal cortex,<sup>7</sup> in the hyperplastic cortex<sup>8</sup> and in cortical tumors,<sup>9</sup> and the claim has been made that it is specifically correlated with modification of sexual characteristics, usually in the direction of masculinization.<sup>10</sup> Others have felt that increases in fuchsinophilia were related rather to age,<sup>11</sup> to cell senescence<sup>12</sup> and to both these factors, with hormonal changes in both male and female.<sup>13</sup>

From the Department of Pathology, Harvard Medical School, Boston, and the Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania, Philadelphia.

1. Zalesky, M.: *Anat. Rec.* **60**:291, 1934.
2. Uotila, U. U.: *Anat. Rec.* **75**:439, 1939.
3. Bourne, G.: *Nature, London* **134**:664, 1934.
4. Bennett, H. S.: Personal communication to the authors.
5. Masui, K., and Tamura, Y.: *Jap. J. Zootech. Sc.* **1**:66, 1924.
6. Hill, W. C. O.: *J. Anat.* **68**:19, 1933.
7. Broster, L. R.: Eight Years' Experience with Adrenal Gland, *Arch. Surg.* **34**:761 (May) 1937.
8. Koster, H.; Goldzieher, M. A.; Collens, W. S., and Victor, A.: *Am. J. Surg.* **13**:311, 1931. Broster, L. R.: *Brit. J. Surg.* **26**:930, 1939.
9. (a) Cahill, G. F.; Loeb, R. F.; Kurzrok, R.; Stout, A. P., and Smith, F. M.: *Surg., Gynec. & Obst.* **62**:287, 1936. (b) Holmes, G.: *Quart. J. Med.* **18**:143, 1925. (c) Levy Simpson, S., and Joll, C. A.: *Endocrinology* **23**:59, 1938. (d) Bourne, G., cited by J. Cancer Research Com., *Univ. Sydney* **7**:34, 1935.
10. (a) Broster, L. R., and Vines, H. W. C.: *The Adrenal Cortex: A Surgical and Pathological Study*, London, H. K. Lewis & Co., Ltd., 1933. (b) Goormaghtigh, N.: *Am. J. Cancer* **38**:32, 1940.
11. Sudds, M. V. N.: *Endocrinology* **26**:895, 1940.
12. Blackman, S. S.: *Bull. Johns Hopkins Hosp.* **78**:180, 1946.
13. (a) Hare, D. C.; Ross, J. M., and Crooke, A. C.: *Lancet* **2**:118, 1935. (b) Goormaghtigh, N.: *Le cortex surrénal humain dans les plaies de l'abdomen et aux périodes intéressantes de la vie sexuelle*, Liège, 1922.

In mice, the disappearance of a transitory zone follows sexual changes very closely,<sup>5</sup> and fuchsinophilia of this zone has been both affirmed<sup>13a</sup> and denied.<sup>14</sup>

Because of the obvious interest in a possible histochemical method of localizing masculinizing hormones, the potentialities of acid fuchsin as a stain for adrenal and other tissues have been reinvestigated.

In the original description, maintaining androgenic specificity for fuchsinophilic tissue,<sup>10a</sup> the staining was described as a "diffuse even coloration of cytoplasm," although in some cases the cytoplasm was stated to be "granular."<sup>10a</sup> In Broster's accompanying illustrations, the fuchsinophilic cells are seen to be filled evenly with fine fuchsinophilic granules on a neutral cytoplasmic background. The stain described as ponceau fuchsin was specified in its composition, staining times were specified, and dichromate pretreatment was recommended. In several respects those disclaiming the specificity of the reaction have failed to be as explicit about their technic as the original authors and have based their conclusions on the staining of cytoplasmic inclusions and not on a diffuse reaction of the entire cell.

In particular, Sudds,<sup>11</sup> in relating the increasing occurrence of fuchsinophilic "granules" to increasing age in the human subject, incidentally verified the contention of Broster and Vines that the human embryonic adrenal cortex goes through a diffuse cytoplasmic fuchsinophilic stage. Blackman,<sup>12</sup> apparently unaware of the dichromate mordanting,<sup>15</sup> felt that eosin, phosphotungstic acid-hematoxylin and iron-hematoxylin all gave identical results. In particular, he found smooth muscle, pancreatic acinar tissue and renal tubular epithelium to stain equally well. Goormaghtigh,<sup>10b</sup> in evaluating virilizing tumors, pointed out that in active tumors "the lipid material undergoes complex changes leading to the formation of . . . fuchsinophil granules which accumulate at one pole of the cell." Sections of similar tissues stained by Dr. F. Melicow show much the same findings.<sup>16</sup>

The correlation of fuchsinophilia and mitochondria has been thoroughly investigated in the adrenal.<sup>17</sup> The consensus is that fuchsinophilic granules exist both in the normal adrenal and in adrenal tumors. They stain with mitochondrial stains and are somewhat more resistant to acid treatment than mitochondria; they are preserved by solution of formaldehyde U. S. P. (formalin\*) or Orth's fluid and are destroyed by Zenker's fixing fluid, and their existence within the cells is obscured by the presence of extractable lipids in the cells, much like chondriosomes.<sup>18</sup>

#### EXPERIMENTAL STUDY

A reevaluation of the properties of the stain originally described by Broster and Vines<sup>10a</sup> was carried out in mouse adrenals at various stages of development, in adrenals of human embryos and in thin smears of adult human blood. Variants of

14. Howard, E.: *Am. J. Anat.* **65**:105, 1939.

15. Foote, N. C.: *Stain Technol.* **8**:101, 1933.

16. Melicow, F.: Personal communication to the authors.

17. (a) Goldzieher, M. A.: *The Adrenal Glands in Health and Disease*, Philadelphia, F. A. Davis Company, 1944. (b) Cowdry, E. V.: *Special Cytology: The Form and Function of the Cell in Health and Disease*, New York, Paul B. Hoeber, Inc., 1928, p. 182. (c) Mulon, P.: *Compt. rend. Soc. de biol.* **68**:872, 1910; (d) **70**:652, 1911. Uotila.<sup>2</sup> Bourne.<sup>2d</sup>

18. Giroud, A.: *Compt. rend. Soc. de biol.* **93**:158, 1925.

staining and tissue pretreatment, particularly with regard to oxidation and lipid extraction, are shown in the accompanying table. The stains selected for comparison were as follows:

1. Broster-Vines "ponceau fuchsin."<sup>19</sup> This technic employs two solutions:

A. Ponceau de xylydine .....	1 Gm.	B. Acid fuchsin .....	1 Gm.
Glacial acetic acid .....	1 cc.	Glacial acetic acid .....	1 Gm.
Distilled water .....	100 cc.	Distilled water .....	100 cc.

Take 2 parts A to 1 part B, stain slides for three minutes, differentiate in 1 per cent phosphomolybdic acid and counterstain with 1 per cent aniline blue for five minutes.

2. Aniline acid fuchsin. Tissues were stained according to Bensley's<sup>20</sup> directions except as specified in the table.

*Variants of Pretreatment and Staining of Tissues\**

Blood Smears	Red Blood Cells *	Eosinophils	White Blood Cells:	
			Cytoplasm	Neutrophils
Control				
Dried in air.....	F+	F+	Pale blue	0
Formaldehyde-fixed .....	F++	F++	Pale blue	0
Dichromate pretreatment—after formaldehyde....	F+++	F+	Dark blue	0
Acetone—after formaldehyde and dichromate....	0	F+	Pale blue	0

Adrenals of Embryos	Red Blood Cells	Fibroblasts	Adrenal Cytoplasm		Consistent Granulation Types
			Fetal	Adult	
Formaldehyde—standard Broster-Vines technic, 1 hr. dichromate .....	F++	Blue	F++	Blue	4
Standard aniline acid fuchsin stain.....	F++	Green	Green	Green	2
Xylydine, no dichromate.....	F++	Blue	F++	F	4
Dichromate overnight, Broster-Vines stain.....	F++	Blue	F++	Blue	4
Acetone, Broster-Vines stain.....	F+	Blue	0	Blue	4
Ether, Broster-Vines stain.....	F++	Blue	0	Blue	4
Acid fuchsin, 1 hr. dichromate.....	F+++	Blue	F++	Blue	4
Bensley, no oxidation.....	F++	Green	Green	Green	2
Bensley, 1 hr. dichromate.....	F+++	F+	F++	F+	2, rare 4
Bensley, dichromate, acetone.....	F+	Green	0	Green	2, rare 4

Adrenals of Mice	Red Blood Cells	Fibroblasts	Adrenal Cytoplasm		
			Transitory Zone	Adult	Granulation
Zenker's fluid, Broster-Vines stain.....	F++	Blue	F++	Blue	None, even color
Helly's fluid, Broster-Vines stain.....	F++	Blue	F+	Blue	2, 4
Orth's fluid, Broster-Vines stain.....	F+	Blue	F+	Blue	2, 4

\* F, F+, F++, F+++ stand for degrees of fuchsinophilia.

3. Pituitary aniline blue stain (Children's Hospital, Boston). Stain with 1 per cent acid fuchsin five minutes. Do not wash.

Mallory's phosphomolybdic acid-aniline blue-orange G. Stain for eight minutes. Wash in distilled water.

4. Phenylhydrazine stain. Dr. Bennett's suggestions were followed, as well as his method.<sup>21</sup> Frozen sections were stained one-half the usual times with Broster-Vines stain and were imbedded in gelatin.<sup>22</sup>

19. Ponceau fuchsin-Grubler was not used.


20. Bensley, R. R. and Bensley, S. H.: Handbook of Histological and Cytological Technique, Chicago, University Chicago Press, 1938, p. 90.

21. Bennett, H. S.: Am. J. Anat. 67:151, 1940.

22. Masson's trichrome stain with preliminary dichromate treatment was used<sup>21</sup> in a few cases. The fuchsinophilia was sometimes comparable to that seen with Vines' technic but was less clearcut and more variable.

## RESULTS

Fuchsinophilia of several types was observed (see figure). Fuchsinophilia of type 4, occurring in the transitory zone in the mouse adrenal, was seen in almost all sections using the Broster-Vines technic. Aniline acid fuchsin was less consistent as a cytoplasmic stain, and it was felt that the structures stained with it coincided in the main closely with known mitochondrial distribution. Many other mouse tissues stained with aniline acid fuchsin showed similar fine fuchsinophilic stippling (type 2).

Type of Fuchsinophilia	Granule Detail	Entire Cell	Cytoplasm	Nucleus	Nucleolus	Granulation	Comment
1			Pale clear blue	Deep red	Inconspicuous	Large, refractile, deep red	See Sudds <sup>11</sup>
2			Pale clear blue	Dark blue	Inconspicuous	Fine perinuclear spray	See Bensley and Bensley <sup>20</sup>
3			Pale clear blue	Dark blue	Deep red	Deep red shred in clear zone	See Goormaghtigh <sup>12b</sup>
4			Finely granular pale red	Dark blue	Deep red	Uniform, diffuse	See Broster <sup>7</sup>
5			Finely granular pale red	Dark blue	Deep red	Large, refractile, deep red	Probably types 1 and 4
6			Coarsely and irregularly pale to dark blue	Deep red	Inconspicuous	Large, irregular deep red fragments	Probably degenerating cells

Types of fuchsinophilia seen in human adrenals stained according to the Broster-Vines technic.

The age distribution of diffuse, finely granular cytoplasmic fuchsinophilia already reported <sup>10a</sup> was confirmed in 20 embryos of various ages.

In both blood smears and embryos the staining was variable but could be closely correlated with the pretreatment (table).

Several characteristics of the fuchsinophilic material were shown in all these sections. It has associated lipoids that can be extracted with lipid solvents. It is most striking after mild oxidation or "mordanting" by oxidizing agents. In extra-adrenal tissues, such as the interstitial cells of the testis and the corpus luteum of the mouse and the rat, it shows an interesting correlation with the ketone-

positive phenylhydrazine-reacting substances, although in fixed tissues subjected to solvent defatting the phenylhydrazine reaction is no longer positive. The substances found to stain most consistently with phenylhydrazine by Bennett<sup>21</sup> presumably were ketosteroids, and were found in tissues in which extractable, biologically active 17-ketosteroids were found. This would include progesterone and corticosterone as well as the androgenic ketosteroids. The location of phenylhydrazine-positive material is entirely limited to areas showing vacuolation in the defatted sections, and the fuchsinophilic substance in many cases lies outside these vacuolations, in the cytoplasm itself. Occasionally, as in type 2 fuchsinophilia, vacuolation about a matrix can be seen, as one observer has described.<sup>10b</sup> That there are lipoproteins in mitochondria and chondriosomes is well established,<sup>22</sup> presumably present as complex coacervates. It would be expected that fat solvent treatment would result in just such residues.

We can define what this fuchsinophilia is only by specifying the type of cell components whose affinity for dye is most striking. In frozen sections, where the fuchsinophilic material was found diffusely distributed intracellularly, we were able to show associations of fuchsinophilia in the cytoplasm and phenylhydrazine staining in vacuoles in testis and corpus luteum as well as in adrenal cortex. In the original report<sup>7</sup> fuchsinophilia in such extra-adrenal cells was pointed out. We have seen fuchsinophilia of type 4 in one functioning interstitial cell tumor in man.

The common location of both phenylhydrazine-positive granules and fuchsinophilic cytoplasm in tissues known to produce ketosteroids of physiological importance is perhaps open to alternative explanations unless the reagents in the acid fuchsin stains giving positive results are closely studied. In attempts to improve staining of bacteria with acid fuchsin by standard methods,<sup>24</sup> the affinity of the dye for the terminal amino groups of lysine<sup>25</sup> has been found to be considerably enhanced by esterification of the carboxyl group.<sup>26</sup> It is difficult to reconcile our results with such a mechanism. Direct diazotization of ketone groups in mammalian tissue with other agents has been carried out recently.<sup>27</sup> Our object, however, has been to analyze the specificity of the acid fuchsin reaction itself.

Strong acids destroy the reaction, and mild oxidizing agents enhance it. Formaldehyde solution U. S. P. (formalin\*) is a specific in the primary fixative. The presence of an aromatic amine (aniline, xyldine) is necessary for the full development of acidophilia in these tissues for acid fuchsin.

A close analogy exists among known organic reactions; the reaction described by Mannich,<sup>28</sup> in which formaldehyde solution U. S. P. with an organic amine

23. Cowdry, E. V.: *Am. Naturalist* **60**:157, 1926. Bensley, R. R., and Gersh, I.: *Anat. Rec.* **57**:217, 1933. Chargaff, E.; Edsall, J. T., and Anson, M. L.: *Advances in Protein Chemistry*, New York, Academic Press, Inc., 1944. Claude, A.: *Science* **91**:77, 1940. Mulon.<sup>17c</sup>

24. Stearn, A. E., and Stearn, E. W.: *Stain Technol.* **5**:17, 1930.

25. Klotz, I. M., and Walker, F. M.: *J. Am. Chem. Soc.* **69**:1609, 1947.

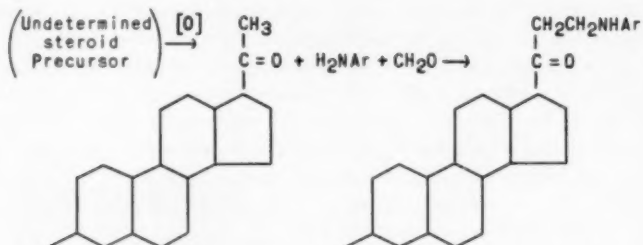
26. Bartholomew, J. W.; Evans, E. E., and Nielson, E. D.: *J. Bact.* **58**:347, 1949.

27. Ashbel, R., and Seligman, A. M.: *Endocrinology* **44**:565, 1949. Camber, B.: *Nature, London* **163**:285, 1949.

28. Mannich, C.: *Arch. d. Pharm.* **255**:261, 1917.

substitutes aminomethyl groups on methyl ketone groups. One would presume that such substitution will result in greater affinity for polysulfonic acid dyes such as has been seen here.

Thus:



It is likely that an activated azo nitrogen in orange G, Biebrich scarlet and nitrazine, as well as xylydine ponceau enter into the reaction in much the same fashion. Of these, one might expect xylydine ponceau to be the most reactive.

It does not seem likely that mild oxidation would affect a methoxy group, and it seems far more likely that an aldehyde-ketone transition is stabilized by oxidation. Once given a ketone group, the pattern becomes that of a substance presumably lipoid, bound to protein, reacting with ketone reagents and capable of amination to yield a substance strongly acidophilic. Solubility experiments would suggest a steroid rather than phosphatide character.

#### SUMMARY

Fuchsinophilia has been studied in murine and human tissues. Reports on fuchsinophilia in the adrenal of the human embryo and in the testis, the adrenal and the corpus luteum of the mouse have been confirmed.

Types of fuchsinophilic reactions are distinguished.

A critical analysis of acid fuchsin methods is made.

An analogy to the reaction described by Mannich is seen, and a ketosteroid-protein complex is suggested as the most likely material staining specifically with these methods.

851 Adelaide Drive.

The problem studied was originally suggested by Dr. S. Burt Wolbach. Drs. Sidney Farber and Arthur T. Hertig made much of the human material studied available.

## CORRELATION BETWEEN CYTOPLASMIC BASOPHILIA AND THE NUCLEIC ACID CONTENT OF THE LIVER

EMMANUEL FARBER, M.D., Ph.D.

DIETER KOCH-WESER, M.D.

PAUL B. SZANTO, M.D.

AND

HANS POPPER, M.D., Ph.D.

CHICAGO

IT IS FAIRLY well established that under physiological circumstances the degree of cytoplasmic basophilia closely correlates with the pentose nucleic acid (ribonucleic acid) content as determined both chemically<sup>1</sup> and by ultraviolet microspectrophotometry.<sup>2</sup> The same relationship has been assumed to be true under pathological conditions.<sup>3</sup>

During studies<sup>4</sup> on the correlation of biochemical and histological alterations of the liver under pathological conditions, it was observed that decreases of cytoplasmic basophilia were not always accompanied by corresponding changes in the nucleic acid content. This prompted a more extensive investigation of this correlation in which the concentration and the amount of total and of pentose and desoxypentose nucleic acids were compared with the degree of basophilia observed in livers following the induction of fatty changes with and without necrosis.

Dr. Koch-Weser is Solomon Foundation Fellow.

Dr. Farber is American Cancer Society Fellow on recommendation of the Committee on Growth of the National Research Council. Present address: Department of Pathology, Tulane University of Louisiana School of Medicine, New Orleans 12, La.

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From the Hektoen Institute for Medical Research and the departments of pathology of Cook County Hospital and Northwestern University Medical School.

1. Brachet, J.: *Enzymologia* **10**:87, 1941; *Ann. New York Acad. Sc.* **50**:861, 1950. Davidson, J. N., and Waymouth, C.: *J. Physiol.* **105**:191, 1946.

2. Caspersson, T.: *Chromosoma* **1**:147, 562 and 605, 1940.

3. (a) Szanto, P. B., and Popper, H.: *Arch. Path.*, this issue, p. 409. (b) Popper, H.; Koch-Weser, D., and Szanto, P. B.: *Proc. Soc. Exper. Biol. & Med.* **71**:688, 1949. (c) McKay, D. G., and Farrar, J. T.: *Cancer* **3**:106, 1950. (d) Drochmans, P.: *Experientia* **3**:421, 1947.

4. (a) Koch-Weser, D.; Farber, E.; Szanto, P. B., and Popper, H.: *Federation Proc.* **9**:336, 1950. (b) Koch-Weser, D.; Szanto, P. B.; Farber, E., and Popper, H.: *J. Lab. & Clin. Med.* **36**:694, 1950.

## MATERIALS AND METHODS

Sixty-five female albino rats, all of the same strain and weighing from 180 to 220 Gm., were used. Prior to the experiment the animals had been fed a commercial diet.<sup>5</sup> All groups were deprived of food for 16 hours before the time of intoxication and thereafter until killed. In one group, carbon tetrachloride (chemically pure) was administered intraperitoneally in a single dose of 0.03 cc. (in 0.3 cc. of liquid petrolatum) per 100 Gm. of body weight. This type of administration produces a highly reproducible central necrosis of the hepatic lobule with surrounding fatty metamorphosis. A second group received *dl*-ethionine (alpha amino-gamma ethylmercaptobutyric acid), a presumed metabolic antagonist of methionine, in which the S-methyl group of the latter is replaced by S-ethyl. This substance rapidly induces fatty metamorphosis of the liver in adult female rats<sup>6</sup> and depresses the rate at which radioactive methionine (S35) and glycine (C14) are incorporated into the proteins of the liver *in vivo*.<sup>7</sup> The ethionine was administered intraperitoneally in aqueous solution (25 mg. per cc.) after neutralization with sodium bicarbonate. The animals received 0.75 mg. of ethionine per gram of initial body weight in three divided doses (one every 2½ hours). A third group, fasted for the same length of time as the experimental animals, served as controls. The animals were killed 24, 48 and 120 hours after the first administration of the compounds. After the liver was weighed, an aliquot for chemical determinations was immediately frozen on solid carbon dioxide. Thin slices of liver were fixed in Zenker-formaldehyde solution (Helly's fluid) and in Carnoy's fluid. In addition to sections stained with hematoxylin and eosin, paraffin sections were stained for basophilic cytoplasmic material with methyl green-pyronine, toluidine blue O and galloyanin-chromalum. The latter two dyes have an affinity for both pentose and desoxypentose nucleic acids and stain them indiscriminately. Pyronine, on the other hand, stains pentose nucleic acid<sup>8</sup> present in the cytoplasm and nucleolus, while methyl green selectively combines with polymerized desoxypentose nucleic acid present in the nucleus. Parallel slides were stained after they had been incubated at 60 C. for three hours in a 0.1 per cent solution of purified ribonuclease buffered with sodium barbital to  $pH$  6.75. Suitable controls were incubated in the same buffer solution without the enzyme. For semiquantitative comparison with the chemical results, the depletion of basophilia (taking into account intensity and extent) was arbitrarily graded by microscopic examination without knowledge of the experimental history or the chemical results.

For the chemical determination of the nucleic acids, the frozen liver was homogenized in ice-cold distilled water, and to suitable aliquots was added an equal volume of ice-cold 10 per cent trichloroacetic acid. The total nucleic acids were determined both by the method of Schneider<sup>9</sup> and by that of Schmidt and Thannhauser.<sup>10</sup> With the former method, the total phosphate was determined<sup>11</sup> after the nucleic acids had been extracted from the washed lipid-free residue with 5 per cent trichloroacetic acid for 20 minutes at 90 C. With the latter method, the total phosphate was determined after the lipid-free residue had been extracted with normal potassium hydroxide. In an additional series of experiments the nucleic acids were fractionated into pentose and desoxypentose nucleic acids by the procedure of Schmidt and Thannhauser. The pentose nucleic acid was determined as pentose (but is expressed in the tables as phosphorus) according to the method of Mejbaum<sup>12</sup> as modified by Miller, Miller and Golden.<sup>13</sup> The desoxypentose nucleic acid was estimated as phosphate in the acid-insoluble residue.

5. This was the dog chow produced by the Ralston Purina Company, St. Louis.

6. Farber, E., Simpson, M. V., and Tarver, H.: *J. Biol. Chem.* **182**:91, 1950.

7. Simpson, M. V.; Farber, E., and Tarver, H.: *J. Biol. Chem.* **182**:81, 1950.

8. Brachet, J.: *Arch. biol.*, Paris **53**:207, 1942.

9. Schneider, W. C.: *J. Biol. Chem.* **161**:293, 1945.

10. Schmidt, G., and Thannhauser, S. J.: *J. Biol. Chem.* **161**:83, 1945.

11. Fiske, C. H., and Subbarow, Y.: *J. Biol. Chem.* **66**:375, 1925.

12. Mejbaum, W.: *Ztschr. f. physiol. Chem.* **258**:117, 1939.

13. Miller, G. L.; Miller, E. E., and Golden, R. H.: *Federation Proc.* **9**:206, 1950.

The total nitrogen (N) was determined by sulfuric acid digestion of a suitable aliquot of liver followed by nesslerization.

Since large changes in liver weight occurred, the nucleic acid values were expressed in both hepatic concentration (mg. per 100 Gm. of wet liver weight) and total amount (mg. per 100 Gm. of initial body weight). The weights of the animals at the beginning of the fasting period were considered the initial body weights.

#### RESULTS

*Histological Investigations.*—In the fasted control animals the distribution of the diffuse and granular basophilic material was similar to that described in fed animals by Deane.<sup>14</sup> However, a general decrease of cytoplasmic basophilia was noted. Staining red with pyronine, blue with toluidine blue and black with gallo-cyanin, the basophilic bodies varied in size and number in the different zones of the hepatic lobule. In the central zone the bodies were large and frequently rod-shaped; in the intermediate zone they were smaller but more numerous; in the peripheral zone, especially in the limiting plate around the portal triads, few basophilic bodies were seen, but instead an intense diffuse basophilia was observed. Similarly, the intracellular distribution varied as described.<sup>14</sup> In some cells the basophilic bodies showed a predominantly perinuclear arrangement; in others they were uniformly distributed. Infrequently they were seen only in the peripheral portions of the liver cells. They were more numerous along the bile capillaries in a palisade-like arrangement. Few of the Kupffer cells and portal histiocytes showed basophilia. The bile duct epithelium revealed some diffuse basophilia (fig. 1 A and B).

Twenty-four hours after carbon tetrachloride intoxication the central third to central half of the liver lobule was necrotic and the nuclei were either absent or showed karyorrhexis or karyolysis. Some cells, mostly on the periphery of the necrotic area, showed hydropic swelling. Basophilia, diffuse or granular, was entirely absent from the necrotic zone except for some in proliferating Kupffer cells and wandering histiocytes. With toluidine blue, a diffuse sky blue hue of the necrotic liver cells was noted. This color, in contrast to the basophilia so far mentioned, was not removed by ribonuclease. The area of central necrosis was surrounded by a zone in which small and large fat droplets were accumulating in the liver cells. In these cells basophilia was revealed only in a small rim around the nucleus and around the fat droplets, producing a foamy network. In the peripheral zone many liver cells contained fine fat droplets in their diffusely basophilic cytoplasm. The Kupffer cells in the peripheral zone were rich in basophilia, as were the histiocytes in the portal areas (fig. 1 C and D).

In rats killed 48 hours after the administration of carbon tetrachloride the necrotic and fatty changes and the cellular infiltration, as well as the degree of basophilia, were essentially the same as in the 24 hour group. In the peripheral zone many mitotic figures and cells with large nuclei containing large nucleoli were seen. In such cells the basophilic material was increased in the nucleoli and around the nuclei. The greatest increase concerned the diffuse basophilia of the limiting membrane (fig. 2 A). This limited increase, however, did not suffice to influence the low grading of over-all basophilia.

14. Deane, H. W.: *Am. J. Anat.* 78:227, 1946.

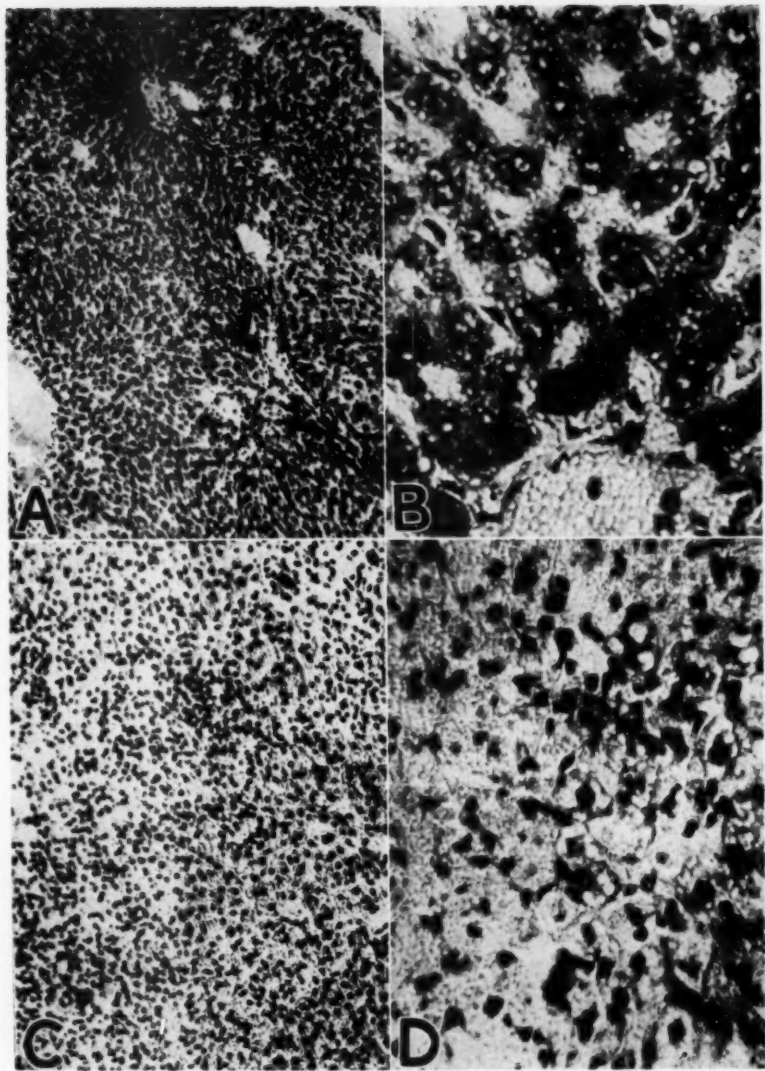


Fig. 1.—*A*, liver of normal rat after 40 hours of fasting ( $\times 110$ ). The cytoplasm of all cells is rich in basophilic material.

*B*, central lobular zone of liver of normal rat after 40 hours of fasting ( $\times 400$ ). Large rod-shaped basophilic granules are distinct and equally distributed in the cytoplasm of the hepatic cells.

*C*, liver of fasting rat 24 hours after administration of carbon tetrachloride ( $\times 110$ ). There are lack of nuclear staining in the central third of the lobule and lack of cytoplasmic basophilia in about three fourths of the lobule.

*D*, border of intermediary and central lobular zone of liver of fasting rat 24 hours after administration of carbon tetrachloride ( $\times 400$ ). In the left half the hepatic cells reveal either lack of nuclear staining or degenerated nuclei, whereas in the right half the nuclei are intact. Basophilic bodies are missing. Note diffuse cytoplasmic basophilia of Kupffer cells and histiocytes. (All slides were stained with methyl green pyronine.)

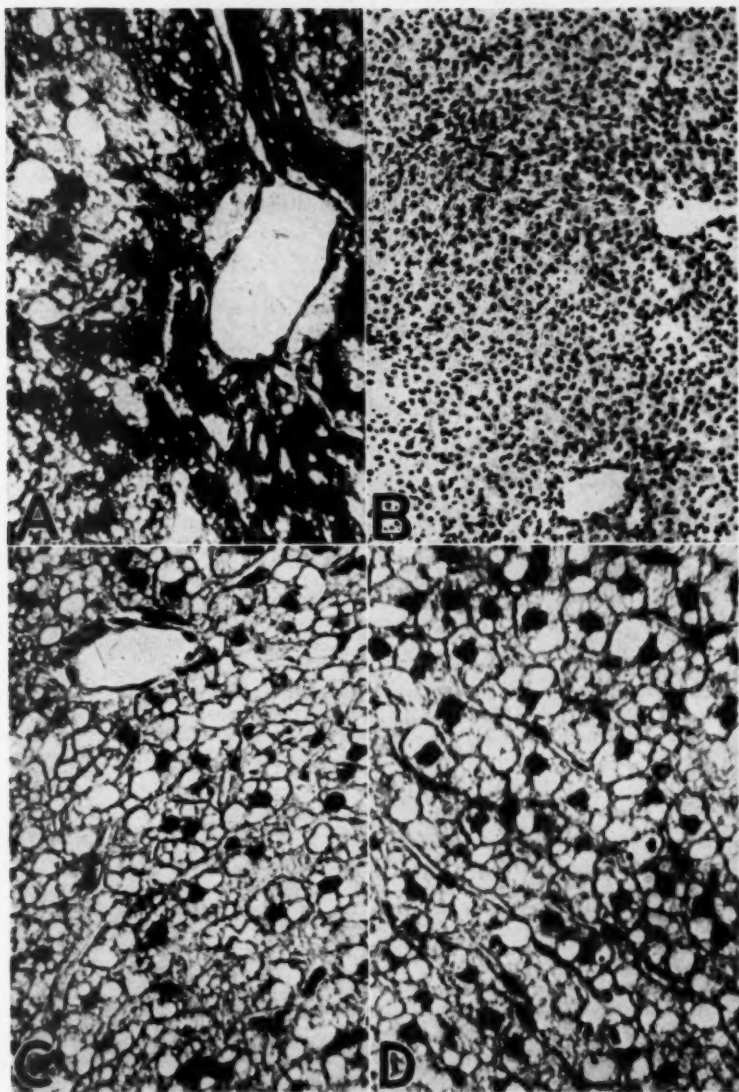


Fig. 2.—A, peripheral zone of liver of fasting rat 48 hours after administration of carbon tetrachloride ( $\times 400$ ). Increased basophilia of the hepatic cells is seen, often arranged around nuclei, even in cells containing fat droplets.

B, liver of fasting rat 24 hours after administration of ethionine ( $\times 110$ ). Note diffuse reduction of basophilia and fatty changes decreasing toward the center.

C, peripheral zone of liver of fasting rat 24 hours after administration of ethionine ( $\times 400$ ). The liver cells contain smaller and larger fat droplets, and basophilic material is only rarely seen either around the nuclei or in the wall of the fat droplets.

D, peripheral zone of liver of fasting rat 48 hours after administration of ethionine ( $\times 400$ ). The fat droplets in the hepatic cells are larger, and basophilia is almost entirely absent. (All slides were stained with methyl green pyronine.)

After 120 hours only a few small residual foci of necrotic tissue were seen in the central zone. The basophilia was essentially the same as that seen in the fasted controls. Mitotic figures were no longer visible. Some residual cellular infiltration was noted.

In rats killed 24 hours after administration of ethionine, the hepatic cells were studded with small and large fat droplets, the larger droplets being predominantly in the peripheral zone of the lobule. The fatty changes showed a decrease toward the center. The basophilic material was definitely reduced and was noted as a thin rim around the nucleus and less clearly around the fat droplets, producing a reticu-

TABLE 1.—Total Nucleic Acids in Livers of Rats Intoxicated with Carbon Tetrachloride and Ethionine (Determined According to Schneider)

Rats	Type	Time at Which Rats Were Killed, Hr.	Total Nucleic Acids, Mg. P per 100 Gm. of	
			Liver	Initial Body Weight
3	Control .....	24	90 ± 9 *	2.39 ± 0.13
5	Carbon tetrachloride .....	24	62 ± 7	2.37 ± 0.30
3	Ethionine .....	24	60 ± 2	2.50 ± 0.23
3	Control .....	48	90 ± 5	2.45 ± 0.30
10	Carbon tetrachloride .....	48	103 ± 11	3.64 ± 0.57
5	Ethionine .....	48	66 ± 16	2.10 ± 0.42
1	Control .....	120	100	3.18
3	Carbon tetrachloride .....	120	104 ± 18	2.22 ± 0.14
3	Ethionine .....	120	107 ± 6	2.43 ± 0.36

\* Standard deviation of the mean.

TABLE 2.—Pentose Nucleic and Desoxypentose Nucleic Acids in Livers of Rats Intoxicated with Carbon Tetrachloride and Ethionine

Rats	Type	Time at Which Rats Were Killed, Hr.	Pentose Nucleic Acid, Mg. P per 100 Gm. of		Desoxypentose Nucleic Acid, Mg. P per 100 Gm. of	
			Liver	Initial Body Weight	Liver	Initial Body Weight
3	Control .....	24	84 ± 2 *	2.08 ± 0.16	23 ± 0.2	0.57 ± 0.02
6	Carbon tetrachloride .....	24	49 ± 8	1.94 ± 0.37	16 ± 2.0	0.62 ± 0.06
3	Ethionine .....	24	50 ± 6	1.98 ± 0.14	16 ± 2.0	0.61 ± 0.08
8	Control .....	48	86 ± 7	1.96 ± 0.17	27 ± 9.0	0.62 ± 0.15
8	Carbon tetrachloride .....	48	70 ± 9	2.73 ± 0.30	24 ± 7.0	0.82 ± 0.27
3	Ethionine .....	48	55 ± 7	1.90 ± 0.14	22 ± 2.0	0.75 ± 0.02

\* Standard deviation of the mean.

lated appearance of the cytoplasm. Basophilic granules were few and were seen only in some cells around the central vein. The limiting plate had few fat droplets and showed diffuse basophilia. In all zones the nuclei and nucleoli were small. The Kupffer cells and the periportal histiocytes revealed increased basophilia (fig. 2B and C).

After 48 hours, more and larger fat droplets were observed. No characteristic nucleolar alterations were noted (fig. 2D).

After 120 hours the picture was variable. Occasionally in the peripheral zone some large fat droplets could be seen. These cells showed diminished basophilia. In the uninvolved cells the basophilia did not significantly differ from that in the fasted controls. Mitoses were rare.

*Chemical Investigations.*—The concentration of the total nucleic acids (mg. per 100 Gm. of wet liver) decreased significantly in both carbon tetrachloride-intoxicated and ethionine-treated animals after 24 hours; the total amount (mg. per 100 Gm. of initial body weight), however, remained unchanged (table 1). The same findings were obtained in the ethionine-treated group after 48 hours. At this interval of time the carbon tetrachloride-intoxicated rats showed, in comparison with the con-

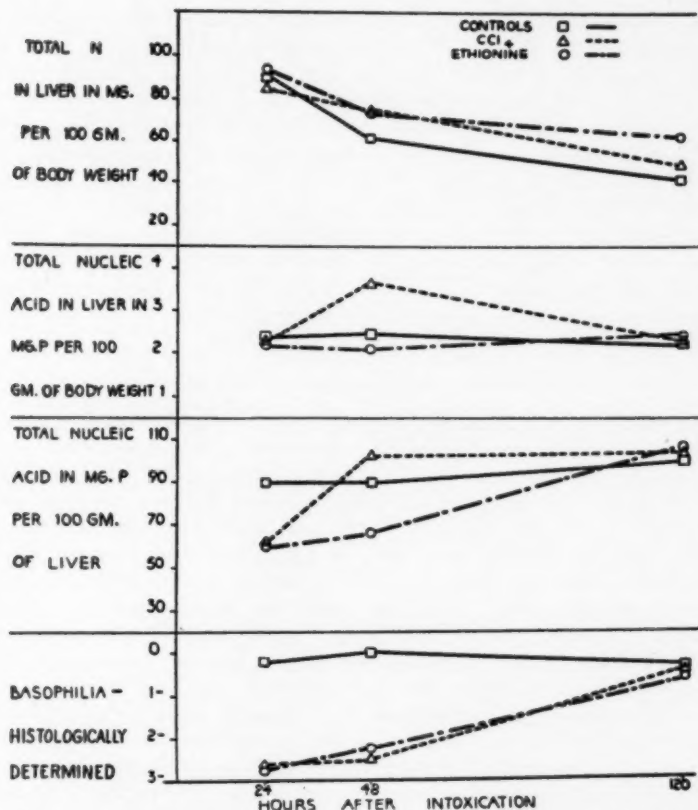


Fig. 3.—The amount of total nitrogen, the amount of total nucleic acids and the concentration of total nucleic acids are compared with the histologically estimated degree of depletion of basophilia in the liver of fasted rats intoxicated with carbon tetrachloride and ethionine.

trols, not only no decrease but an increase of the amount of total nucleic acids. The concentration was equal to or almost equal to that of the controls. After 120 hours all three groups had almost equal concentrations and amounts of total nucleic acids in their livers.

In an additional series of experiments the values for pentose nucleic acid mirrored closely those for total nucleic acids (table 2). At 24 hours after ethionine and carbon tetrachloride administration, and at 48 hours after ethionine, the hepatic

concentration was considerably reduced while the total amount in the liver showed no significant change from the controls. At 48 hours in the carbon tetrachloride-treated rats the concentration was almost the same as in the controls, though the total amount had increased significantly. The total amount of desoxypentose nucleic acid was not decreased at 24 hours; after 48 hours it showed a variable response. The concentration of desoxypentose nucleic acid decreased in both groups after 24 hours.

The chemical values (both concentration and total amount) of total nucleic acids and of total nitrogen of the liver were correlated graphically (fig. 3). The total nitrogen (expressed in amount) dropped with time (because of fasting) equally in the controls and in the experimental animals. The variations in the concentration of total nucleic acids paralleled closely the histochemical grading of the basophilia at 24 hours in both carbon tetrachloride-intoxicated and ethionine-treated rats, both showing a considerable decrease. At 48 hours, however, a notable discrepancy occurred. While the degree of basophilia was markedly decreased, the concentration of total nucleic acids as determined chemically equaled the control values. The total amount of total nucleic acids showed no correlation with the degree of basophilia. The difference was greatest in the carbon tetrachloride group killed after 48 hours. Despite a marked decrease in cytoplasmic basophilia, the livers of these animals contained more total nucleic acids than did the controls.

#### COMMENT

The most important result of this study is the discrepancy found between the degree of visible cytoplasmic basophilia (considered to be due to pentose nucleic acid) and the chemical values for the total and pentose nucleic acids. In a fatty liver without necrosis the degree of basophilia notably decreases coincident with a drop in the concentration of pentose nucleic acid. The total amount of this acid in the liver, however, remains unchanged. In a fatty liver with extensive necrosis the basophilia disappears completely from the necrotic area and becomes markedly reduced in the fatty zones. Twenty-four hours after intoxication the hepatic concentration is reduced while the total amount is normal. With the appearance of regeneration, even though the over-all basophilia is minimal, the concentration of pentose nucleic acid increases to that of the controls, while the total amount of the acid is above normal. The control values for total nucleic acids, pentose nucleic acid and desoxypentose nucleic acid found in this investigation agree well with those reported in the literature for both the Schneider and Schmidt-Thannhauser methods.

That basophilia disappears from necrotic and fatty zones of the liver after carbon tetrachloride intoxication,<sup>15</sup> and from other experimental, as well as from human, fatty livers,<sup>16</sup> is well recognized. Unexplained differences between the degree of basophilia and the values for chemically determined "labile cytoplasm" were reported in experimental carbon tetrachloride intoxication by Campbell and Kosterlitz,<sup>17</sup> who determined the sum of the protein plus phospholipid plus nucleic acids. More recently, Koch-Weser, Szanto, Farber and Popper<sup>4b</sup> found that, despite differences

15. (a) Cameron, G. R., and Karunaratne, W. A. E.: *J. Path. & Bact.* **42**:1, 1936. (b) Rosin, A., and Doljanski, L.: *Proc. Soc. Exper. Biol. & Med.* **62**:62, 1946. (c) Williams, W. L., and Greenberg, A. J.: *Federation Proc.* **9**:348, 1950.

16. Rosin and Doljanski.<sup>15b</sup> Williams and Greenberg.<sup>15c</sup> Szanto and Popper.<sup>3a</sup>

17. Campbell, R. M., and Kosterlitz, H. W.: *Brit. J. Exper. Path.* **29**:149, 1948.

in the degree of basophilia in sections of livers of rats which had been intoxicated with carbon tetrachloride 48 hours earlier with and without prior protection of vitamin B<sub>12</sub> concentrate, no corresponding changes could be demonstrated in the amounts of nucleic acids. The present study indicates that the discrepancy concerns mainly the pentose nucleic acid.

It is difficult to investigate the causes of this discrepancy in carbon tetrachloride-treated animals because in these the changes in the liver are complex, owing to the zonal character of the necrotic, fatty and regenerative changes. Theoretically, losses of chemical substances in the central and intermediate zones might be compensated for by increases in the intact or regenerating peripheral zone. Increments due to the infiltration of basophilic histiocytes and to the proliferation of Kupffer cells may also help to counteract any decreases in liver cells. These phenomena are difficult to assess quantitatively.

The rise in the total amount of pentose nucleic acid in the carbon tetrachloride group after 48 hours is probably due to the associated regeneration as indicated by mitoses, increased basophilia and large nucleoli in the peripheral zone.<sup>17</sup> Increased basophilia has been reported to accompany regeneration in the liver.<sup>18</sup> In rats, after removal of 70 per cent of the liver the regenerating portion shows a maximal increase in pentose nucleic acid concentration of about 60 per cent.<sup>19</sup> In carbon tetrachloride-damaged livers after 48 hours, even such an increase due to regeneration in the relatively narrow peripheral zone would not suffice to make up for an almost complete loss of pentose nucleic acid in the central and intermediate zones, where little or no basophilia is seen. This, coupled with the unchanged total amount at 24 hours, when no signs of regeneration are evident, make it highly probable that the central and midzonal areas still contain considerable quantities of pentose nucleic acid despite the absence or frank decrease in cytoplasmic basophilia.

The observations on the ethionine-treated rats are not subject to the same uncertainties, since the liver shows a diffuse loss of basophilia without areas or cells capable of compensating and without evidence of regeneration but with a normal total amount of total nucleic acids and pentose nucleic acid.

A full explanation of the basic discrepancy appears not possible from the information available so far. Several factors can be considered: Some basophilic material may be removed during the preparation of the slides of the liver.<sup>20</sup> However, the fixatives used should precipitate nucleic acids, and sections of liver prepared by dry-freezing revealed the same depletion of basophilia as those fixed in Carnoy's solution.<sup>21</sup>

The most obvious factor for the decrease in the visible basophilia is an increase in the size, both area and volume, of the liver cells. Since the intensity of staining observed either visually or photometrically is a function of the concentration of dye and not of the total amount within any cell, any significant increase in the volume of a cell results in a decrease in the degree of observable stain. Stowell and Lee,<sup>22</sup>

18. Stowell, R. E.: *Cancer* **2**:121, 1949. Brachet, J.; Jeener, R.; Rossell, M., and Thonet, L.: *Bull. Soc. chim. biol.* **28**:460, 1946.

19. Novikoff, A. B., and Potter, V. R.: *J. Biol. Chem.* **173**:223, 1948.

20. Kosterlitz, H. W.: Personal communication to the authors.

21. Lagerstedt, S.: *Acta anat. (supp. 9)* **7**:1, 1949.

22. Stowell, R. E., and Lee, C. S.: *Histochemical Studies of Mouse Liver After Single Feeding of Carbon Tetrachloride*, *Arch. Path.* **50**:519 (Nov.) 1950.

by cell measurements, have established that large increases in the size of liver cells occur in mice intoxicated with carbon tetrachloride. Furthermore, the total liver weights of rats increase by about 50 per cent subsequent to the administration of either ethionine or carbon tetrachloride.<sup>4a</sup> This rise precedes the appearance of stigmas of regeneration by about 36 hours.<sup>4a</sup> This dilution factor is clearly shown by comparing the concentration with the total amount of pentose nucleic acid (or total nucleic acid) in any single experimental group. It appears questionable, however, whether it suffices quantitatively. Moreover, reduction of basophilia of the liver cells in the central zone of the lobule has been described in the early stages of carbon tetrachloride intoxication<sup>23</sup> before necrosis, fatty changes or liver enlargement become apparent.<sup>4a</sup>

It cannot be excluded, therefore, that in the fatty, necrotic and even preneurotic cell the physical or the chemical state of pentose nucleic acid is changed so that it does not exhibit basophilia but is still demonstrable with the chemical method used. The results of this investigation indicate that basophilia may disappear without change in nucleic acid content and that it is dangerous to draw conclusions about the pentose nucleic acid content of liver cells under pathological conditions from a microscopic examination of liver sections. It has to be proved whether or not the absence of the basophilic reaction, even without alteration of the chemical values for pentose nucleic acid, indicates an impairment of the function ascribed to pentose nucleic acid.

#### SUMMARY

The degree of visible basophilia in sections of liver stained with methyl green pyronine, toluidine blue and galloxyanin-chromalum was compared with quantitative determinations of hepatic concentration and amount of total nucleic acids and pentose and desoxypentose nucleic acids in fasted female rats intoxicated with carbon tetrachloride and ethionine.

Twenty-four hours after administration of carbon tetrachloride there was a loss of basophilia in the central and intermediate zones of the hepatic lobules and 24 hours after that of ethionine there was a diffuse decrease of basophilia in the liver. These events were associated with a decrease in the concentrations of total, pentose and desoxypentose nucleic acids expressed in milligrams per 100 Gm. of wet liver weight but with no change in the hepatic amount expressed in milligrams per 100 Gm. of initial body weight.

Forty-eight hours after administration of ethionine the findings were the same as at 24 hours. Forty-eight hours after administration of carbon tetrachloride, with about the same decrease of basophilia in the large fatty and necrotic areas, regenerative changes with locally increased basophilia appeared in the peripheral zone. The total nucleic acid and pentose nucleic acid concentrations became equal to, and the total amounts increased over, those in the control animals.

This study demonstrates that cytoplasmic basophilia as determined by microscopic study of stained slides does not necessarily give any indication of the actual pentose nucleic acid content of the liver under pathological conditions.

23. Rosin and Doljanski.<sup>13b</sup> Stowell and Lee.<sup>22</sup>

## BASOPHILIC CYTOPLASMIC MATERIAL (PENTOSE NUCLEIC ACID)

Distribution in Normal and Abnormal Human Liver

PAUL B. SZANTO, M.D.

AND

HANS POPPER, M.D., Ph.D.

CHICAGO

THE BASOPHILIC substance in the cytoplasm and nucleus of cells of various organs had been tentatively identified as pentose nucleic acid (ribonucleic acid) because it could be digested by the specific enzyme ribonuclease<sup>1</sup> and showed specific absorption of ultraviolet rays.<sup>2</sup> The pentose nucleic acid is, in most instances, bound to protein. It is to be distinguished from the desoxypentose nucleic acid of the nuclear chromatin, which gives the Feulgen reaction. The phosphoric acid radical of both nucleic acids is responsible for the basophilia, since the other intracellular acid valences are removed during the preparation of tissue sections; intracellular basophilia is almost specific for nucleic acids. Much evidence has been brought forward, especially by Caspersson<sup>2a</sup> and Brachet,<sup>1</sup> that pentose nucleic acid plays an important part in the cytoplasmic protein formation. Since formation of proteins, especially those of the serum, is one of the most important functions of the liver, the presence and the distribution of cytoplasmic basophilia in the liver may offer a histological criterion of the functional status of the liver, or at least some aspects of it. Such a criterion should improve the so far hazy correlation of the histological changes in the liver and the clinical and laboratory findings. In addition, the histological observations might possibly broaden the knowledge of the role played by the liver in protein metabolism. Moreover, the distribution of the basophilic material may offer added criteria for the histological diagnosis of hepatic disease. It appeared advisable, therefore, to study the distribution of the basophilic material in the human liver. This study, which was started independently and without knowledge of the recent study by McKay and

From the Department of Pathology and the Hektoen Institute for Medical Research of the Cook County Hospital and the Department of Pathology of Northwestern University Medical School.

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1. Brachet, J.: *Chemical Embryology*, New York, Interscience Publishers, Inc., 1950.

2. (a) Caspersson, T.: *Cell Growth and Cell Function: A Cytochemical Study*, New York, W. W. Norton & Company, Inc., 1950. (b) Pollister, A. W., and Ris, H.: *Cold Spring Harbor Symp. Quant. Biol.* **12**:147, 1947.

Farrar,<sup>3</sup> confirms, in general, their findings. However, in the present study special emphasis was laid on the findings in liver biopsy specimens in which agonal and postmortem changes<sup>4</sup> do not obscure the picture.

Two problems arise in such a study: (1) Is the pentose nucleic acid actually related to protein synthesis and (2) does the histologically visible basophilia reflect the pentose nucleic acid content of the organ? The hypothesis as to the role of pentose nucleic acid in protein formation is based on the fact that it occurs in high concentration in cells which produce large amounts of protein, e. g., nerve cells,<sup>5</sup> pancreatic cells,<sup>6</sup> hepatic epithelial cells,<sup>7</sup> basophilic cells of the anterior lobe of the pituitary gland,<sup>8</sup> lymphocytes and plasma cells,<sup>9</sup> trophoblasts,<sup>10</sup> embryonal cells<sup>1</sup> and cancer cells.<sup>11</sup> In addition, various physiological observations, recently summarized by Brachet,<sup>12</sup> support this hypothesis; to list some examples, the basophilia of the pituitary gland increases during secretion stimulated by administration of estrogen or androgen,<sup>13</sup> and the P32 activity of the nucleoprotein fraction of yeast decreases during growth, suggesting that the pentose nucleic acid serves as energy donor for protein synthesis.<sup>14</sup> Close correlation has been demonstrated between protein formation and the pentose nucleic acid of the cytoplasm and nucleolus of the liver cell.<sup>15</sup> Nevertheless, so far the biochemical nature of the link between pentose nucleic acid and protein synthesis has not been elucidated, and to date the possibility cannot be fully excluded that pentose nucleic acid is present in the listed locations as result rather than as cause of protein formation.

Independent of the outcome of this argument, it may suffice for the purpose of this study to consider the presence of pentose nucleic acid as a morphological sign of the location of protein synthesis. Opie assumed a close relationship between pentose nucleoprotein and mitochondria. In ultracentrifugation studies<sup>12</sup> pentose nucleic acid was found mainly in the large granular (mitochondrial) and submicroscopic granular (microsomal) fraction and only little in the supernatant fluid. In slides of fixed liver the basophilia appears mostly granular. Therefore, even if the entire granular fraction may be required for protein synthesis, basophilia in tissue sections remains still an index of the cellular activity (as far as protein synthesis is concerned).

In answer to the second question, there is little doubt that the cytoplasmic basophilic bodies indicate, under normal circumstances, the amount of pentose

3. McKay, E. G., and Farrar, J. T.: *Cancer* **3**:106, 1950.

4. Popper, H.: Significance of Agonal Changes in Human Liver, *Arch. Path.* **46**:132 (Aug.) 1948.

5. Gersh, I., and Bodian, D.: *J. Cell. & Comp. Physiol.* **21**:253, 1943.

6. Davidson, J. N.: *Symp., Soc. Exper. Biol.* 1947, no. 1, p. 77.

7. Deane, H. W.: *Am. J. Anat.* **78**:227, 1946.

8. Dempsey, E. W., and Wislocki, G. B.: *Am. J. Anat.* **76**:277, 1945.

9. Wislocki, G. B., and Dempsey, E. W.: *Anat. Rec.* **96**:249, 1946.

10. Wislocki, G. B., and Dempsey, E. W.: *Am. J. Anat.* **78**:181, 1946.

11. Caspersson, T., and Santesson, L.: *Acta radiol.*, 1942, supp. 46, p. 1.

12. Brachet, J.: *Ann. New York Acad. Sc.* **50**:861, 1950.

13. Herlant, M.: *Arch. biol., Paris* **54**:225, 1943. Desclin, L.: *Compt. rend. Soc. de biol.* **133**:457, 1940.

14. Spiegelman, S., and Kamen, M. D.: *Science* **104**:581, 1946.

15. Lagerstedt, S.: *Acta anat. (supp. 9)* **7**:1, 1949.

16. Opie, E. L.: *J. Exper. Med.* **85**:339, 1947.

nucleic acid present. Recent investigations<sup>17</sup> showed, however, that in livers with experimental fatty metamorphosis or with centrolobular necrosis the chemically demonstrable content of pentose nucleic acids may not be diminished although the basophilia is definitely reduced or absent. This suggests that for the basophilic reaction not only the presence but probably also the physical-chemical state (possibly the protein combination) of the nucleic acids is important. In this sense the basophilia may be, under the mentioned abnormal circumstances, a better index of integrity and vitality of the liver than the chemical analysis for nucleic acid.

#### MATERIAL AND METHOD

The material of this study consists of 166 biopsy specimens (from 151 patients) partly obtained by punch and partly by laparotomy. Usually Carnoy's fluid, with or without addition of acetic acid, was used as fixative; in about one fifth of the cases Zenker's fluid (with acetic acid) was used. Carnoy's fluid preserves the basophilic bodies in about the same shape as they are seen by ultraviolet photomicrography in sections fixed by the freezing-vacuum technic.<sup>18</sup> The addition of glacial acetic acid, which improves the penetration of the fixative solution, is unnecessary because of the small size of the biopsy specimen obtained by needle aspiration. Autopsy material of 312 livers fixed in Carnoy's fluid was also studied. In addition, livers of mice and rats subjected to various injuries, such as carbon tetrachloride or allylformate intoxication, were examined.

Paraffin sections were stained with Unna-Pappenheim's methyl green pyronine, toluidine blue O, thionine (Windle's<sup>19</sup> modification), Ehrlich's hematoxylin-eosin and, frequently, with Giemsa stain and galloyanin-chromalum. For the purpose of identifying the basophilic bodies as pentose nucleic acid, methyl green-pyronine and galloyanin preparations were prepared with and without digestion. The latter was carried out at 60 C. for three hours in a 0.1 per cent ribonuclease solution buffered with sodium barbital to  $p_H$  6.75.

#### RESULTS

*Distribution of Basophilic Bodies Under Physiological Circumstances.*—The normal liver cells contained numerous basophilic bodies in their cytoplasm (fig. 1A). The size and the number of these bodies, however, showed physiological variations in the various zones of the lobule in humans and animals. In the central zone the basophilic bodies appeared as well-defined elongated, rod-shaped or comma-shaped structures, many reaching 3 microns in length and 1.5 microns in width. In the intermediate zone the basophilic bodies were smaller and finer. In the peripheral zone, about the portal triads especially, a diffuse cytoplasmic basophilia was observed, and the basophilic bodies were either completely absent or were small and indistinctly outlined, or they appeared as sparse fibers measuring up to 4 microns in length. Generally, the basophilic bodies were large, better defined and more numerous in the central and intermediate zones than in the peripheral zone. The concentration of the basophilic material appeared, however, higher in the peripheral than in the central and the middle zone.

Within the hepatic cells the basophilic bodies frequently showed a predominantly perinuclear arrangement; in some cells, however, they appeared uniformly distributed in the cytoplasm, and infrequently they were noticeable only peripherally in the cells. The distribution of the basophilic bodies was also related to the

17. Farber, E.; Koch-Weser, D.; Szanto, P. B., and Popper, H.: Arch. Path., this issue, p. 399.

18. Lagerstedt, S.: Acta anat. 2:121, 1947.

19. Windle, W. F.; Rhines, R., and Rankin, J.: Stain Technol. 18:61, 1943.

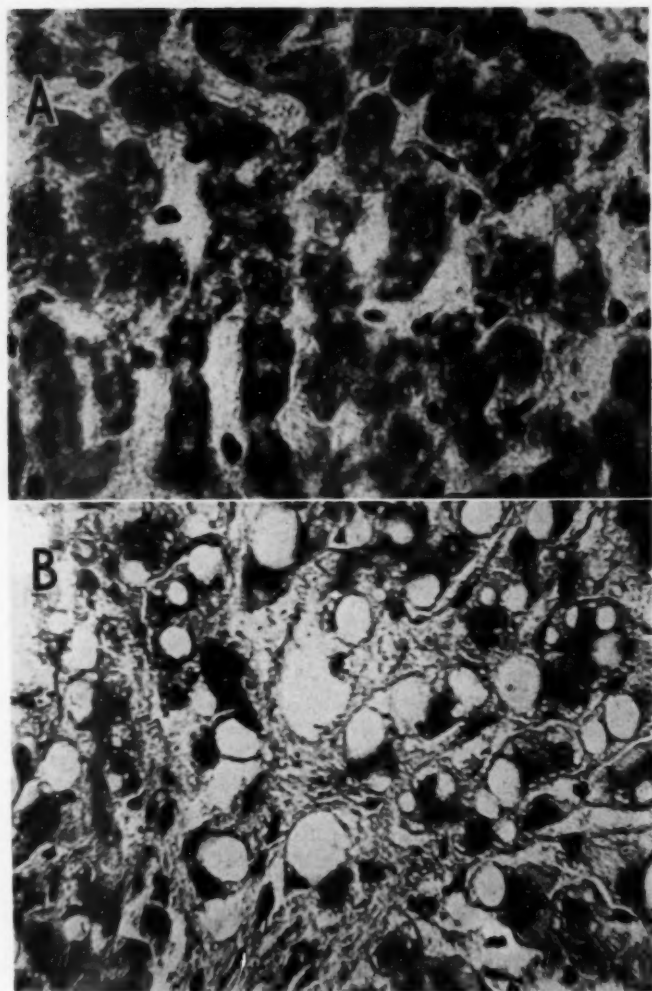


Fig. 1.—*A*, normal liver. Basophilic bodies are present in the cytoplasm of all liver cells (methyl green-pyronine stain;  $\times 400$ ).

*B*, fatty metamorphosis. The hepatic cells show large vacuoles and contain little or no cytoplasmic basophilic material except around the nuclei (methyl green-pyronine stain;  $\times 400$ ).

bile capillaries, along which they were often larger and more numerous, or the diffuse basophilia was more prominent, than elsewhere in the cells. The zone of the hepatic cell midway between nucleus and cell membrane, where glycogen frequently occurs in high concentration, revealed little basophilia.

From the point of view of the relation borne by the basophilic bodies to the nuclei and nucleoli, four types of liver cells have been distinguished. Type 1 showed uniform distribution of the basophilic bodies, a small nucleus and one or more small nucleoli; in type 2 the nucleus was vesicular and contained one or more distinct fair-sized nucleoli and the distribution of the basophilic bodies was not characteristic; the nucleus of type 3 was large, vesicular, and contained one or more distinct large-sized nucleoli and the basophilic bodies showed a perinuclear distribution. The nucleus and the nucleolus of type 4 were small, and the cytoplasm contained no or only few basophilic bodies. In the normal liver type 1 predominates throughout the lobule.

The epithelial cells of the bile ducts contained little or no pentose nucleic acid. The cytoplasm of the Kupffer cells was practically free of basophilic bodies in normal human and mouse livers but showed some basophilia in rat livers.

After the basophilic substance had been digested with ribonuclease, the cytoplasmic and nucleolar basophilia disappeared completely while the nuclear basophilia was only slightly reduced. The digestion, however, did not result in empty spaces; it only reversed the affinity of the clumps from basophilia to acidophilia, owing to the remaining basic protein components of the cytoplasmic pentose nucleoproteins. The nucleic acid component of the nucleoprotein determines the basophilic staining property of the basophilic bodies, and after its hydrolysis the remaining proteins exhibit an acidophilic reaction.

*Distribution of Basophilic Bodies Under Pathological Circumstances.*—Fatty metamorphosis: In liver cells containing small fat droplets the basophilic bodies of the remaining intact cytoplasm are normal or decreased in size and number. Frequently, the fat droplets are so small that they appear to correspond with the mitochondria described by Opie<sup>16</sup> as small cytoplasmic structures—many, but not all of them, giving the staining reaction of mitochondria. The small fat droplets are surrounded by a rim of diffuse basophilia. The larger fat droplets seem to originate from the confluence of smaller ones situated originally within the mitochondria. The basophilic bodies around the large fat droplets are usually notably decreased. However, even in the latter instance a narrow basophilic rim surrounds the flattened nucleus displaced to one side of the cell (fig. 1 B). Pronounced decrease of the cytoplasmic basophilia was observed in the diffuse fatty metamorphosis found at autopsy in 12 cases of alcoholism (fatty liver without cirrhosis).

*Hydropic Degeneration:* In hydropic degeneration the basophilia around the cytoplasmic vacuoles appeared diffuse in distribution and decreased in amount or was completely absent; this was the case even in instances in which the vacuoles were small.

*Coagulation Necrosis:* Clumps of coagulated cytoplasmic protein—e. g., the circumscribed hyaline bodies described by Mallory in alcoholic cirrhosis and the more diffuse intracellular coagulation necrosis of infectious hepatitis (similar to the Councilman bodies in yellow fever)—were completely devoid of basophilic bodies. In anuclear remnants or fragments of the liver cells as they were seen in

the zonal (central) necrosis of toxic hepatitis, the basophilic bodies were completely absent. This was also indicated by the frank eosinophilia of the involved cells or cell fragments in the routine hematoxylin-eosin sections.

**Pigment Deposition:** In liver cells which contained large amounts of wear and tear pigments the basophilic bodies were absent in the area of pigment deposition, but they were preserved in the rest of the cells. Also, in uncomplicated hemosiderosis (two autopsy cases) the basophilic bodies were unchanged in the pigment-free part of the liver cells. Similarly, the presence of small amounts of bile pigment within the liver cells was not necessarily associated with decrease of the basophilic bodies. In cells containing large amounts of fine granular bilirubin the basophilic bodies were usually decreased in number and size. If the cytoplasm of the liver cells appeared diffusely imbibed with bile pigment, no basophilic bodies could be demonstrated. The presence of bile pigment casts within the bile capillaries was not necessarily associated with a decrease of the basophilic bodies in the surrounding liver cells. Indeed, in many cases of obstructive jaundice the basophilic zone surrounding the bile capillaries was wider and basophilia was more intense than normal (fig. 2A).

**Atrophy:** Brown atrophy of the liver cells as observed in 30 necropsy specimens was, as a rule, associated with an apparent increase of the basophilia of the shrunken liver cells. Only a few cells around the central veins with large amounts of lipofuscin contained less basophilic bodies than normal. Liver cells compressed by carcinomatous nodules revealed in 17 of 21 studied necropsy cases an increase in basophilic bodies, except those which showed fatty changes. In the remaining four instances the basophilic bodies were decreased throughout the entire liver, including the compressed areas. In 59 necropsy cases of chronic passive congestion the compressed liver cells in the centers of the lobules showed, as a rule, an increase in basophilic bodies; however, the necrobiotic cells of the central zones were completely free of it and appeared eosinophilic. In two cases of amyloidosis the basophilic bodies of the compressed liver cells were increased and only in disintegrating cells or cell fragments decreased. In a third case of amyloidosis the basophilic bodies were decreased.

**Regeneration:** Regenerating liver cells, either scattered or in nodules, revealed, as a rule, a marked increase of basophilic bodies. They had a large nucleus, rich in desoxyribonucleic acid. The nucleoli were also large. In the proliferating small bile ducts, which are often not easily differentiated from regenerating or atrophic liver cell rows (especially in cirrhosis), the basophilic bodies of the cytoplasm were markedly increased. In proliferated septal or interlobular bile ducts of typical appearance the basophilic bodies did not exceed the small amount found in normal bile ducts. In two small bile duct adenomas examined the basophilic bodies were not increased.

**Hepatocellular Carcinoma:** In four cases the basophilic bodies of the tumor cells were also increased with the exception of the necrotic carcinoma cells, which were completely deprived of cytoplasmic basophilia and showed characteristic eosinophilic cytoplasm. Some of the tumor cells possessed large nucleoli in large nuclei, others small nucleoli in relatively large nuclei.

**Mesenchymal Cells:** The cytoplasm of resting Kupffer cells was practically free of basophilic bodies; such bodies were noted in the mobilized, proliferating Kupffer cells. This cytoplasmic basophilia was especially prominent in Kupffer cells which

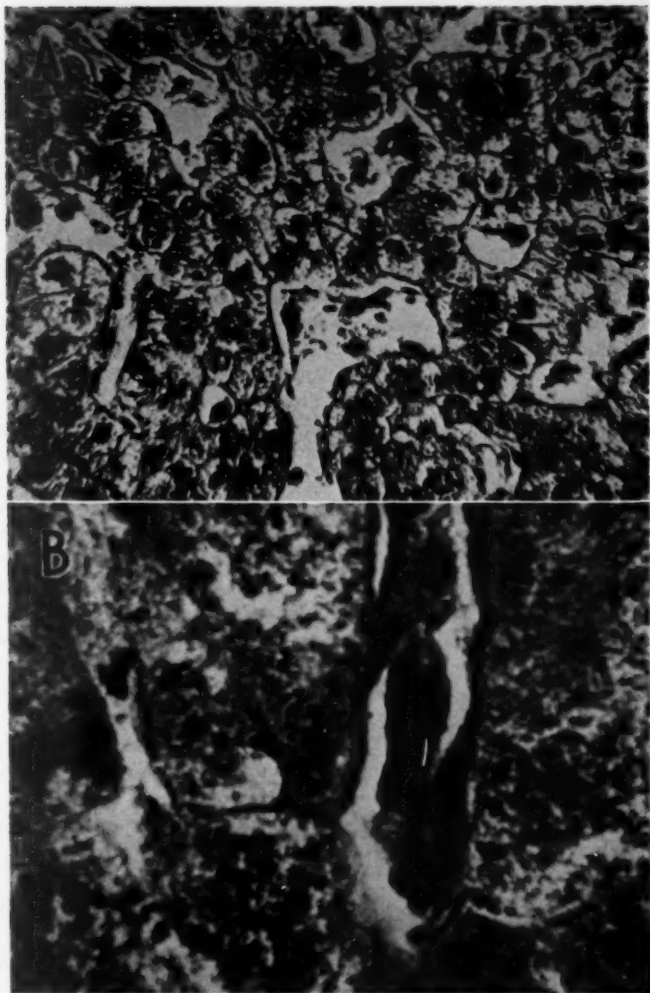


Fig. 2.—*A*, extrahepatic biliary obstruction due to calculus. There are a normal distribution and almost a normal amount of the cytoplasmic basophilic material in the liver cells (methyl green-pyronine;  $\times 400$ ).

*B*, infectious hepatitis. The liver cells show reduction of the cytoplasmic basophilic bodies. The Kupfer cells are rich in basophilic material (methyl green-pyronine stain;  $\times 1,000$ ).

were detached from the sinusoidal lining and floating as spheroid bodies in the lumen. Kupffer cells containing much phagocytosed material like hemosiderin and bilirubin were free of basophilic bodies. Histiocytes and plasma cells and fibroblasts containing basophilic bodies occurred in the periportal field, their presence being usually combined with active cellular infiltration.

*Variations of Basophilic Body Distribution in Hepatic Diseases.*—In keeping with the general alterations of the cytoplasmic basophilia just described, the pattern of its distribution varied in the different hepatic diseases, and in some cases these variations may assume diagnostic significance.

*Infectious Hepatitis:* In seven instances of the fulminant form of infectious hepatitis the basophilic bodies were notably decreased or absent. They were entirely missing from cytoplasm of the hepatic cells which revealed a diffuse hyaline "necrosis" (so-called Councilman bodies). The cell cords near the triads which resemble proliferating bile ducts contained a considerable amount of basophilic bodies. The mesenchymal elements (histiocytes and mobilized Kupffer cells) which crowded the denuded lobular framework and also the portal triads were usually rich in basophilic bodies in contrast to the few remaining liver cells. In 17 biopsy specimens from 14 patients with viral hepatitis a reduction of the basophilic bodies was noted which was parallel to the degree of liver cell damage. This reduction was, in some cases, focal, and in others diffuse but with some central predominance. Scattered hepatic cells with homogeneous eosinophilic cytoplasm were entirely free of basophilic bodies. In some of these cells mitochondria were also absent (eosinophilic necrosis) while in others they were still preserved. Also, in the biopsy specimens, the Kupffer cells within the lobule (fig. 2B) and the mesenchymal cells in the portal triads showed usually distinct cytoplasmic basophilia. Cells with evidence of mitotic and amitotic regeneration revealed increase of the basophilic bodies. The hepatic cells showed a normal or increased basophilia during and after clinical recovery. However, the increased basophilia of the Kupffer cells and the even more increased basophilia of histiocytes in the portal triads persisted, in some cases long after the clinical picture had returned to normal.

*Toxic Hepatitis:* At autopsy in 22 cases of jaundice in which extensive central necrosis indicated the presence of toxic hepatitis (two of them due to substances established as toxins, such as carbon tetrachloride and arsenic, respectively) basophilic bodies were absent or decreased in the necrotic zone and its immediate vicinity. There was a gradual transition from cells with normal or even increased content of basophilic bodies on the periphery of the lobule to cells with intact nuclei but with diminution or absence of basophilic bodies in the cytoplasm; the latter were more or less eosinophilic and might show vacuolation caused by either hydropic degeneration or fatty metamorphosis. In the central zone the anuclear cell remnants or fragments were free of basophilic bodies. The intense eosinophilia in areas of coagulation necrosis in routine hematoxylin-eosin section indicates already the absence of cytoplasmic pentose nucleic acid. In 29 biopsy specimens from 29 patients the basophilic bodies were decreased in the central part of the lobule, their disappearance paralleling the degree of liver cell damage. The alterations were of similar character but of far less intensity than those in the autopsy specimens. In contrast to viral hepatitis, the Kupffer cells showed only occasionally an increase of basophilia; the same holds true for the mesenchymal cells of the portal triads

which showed little tendency toward proliferation. In 12 cases of acute coronary infarction and nine cases of uremia in which extensive central necrosis of the hepatic lobules was revealed, the basophilic bodies were absent from the necrotic zone as well as from the otherwise intact surrounding zone. In three cases of myeloid leukemia scattered, apparently necrotic liver cells were free of cytoplasmic basophilic bodies. Similarly, acidophilic degeneration and necrosis of scattered liver cells have been observed in three cases of Hodgkin's disease; the eosinophilic degenerated liver cells were more numerous in the central zone.

**Liver Cell Damage Due to Extrahepatic Biliary Obstruction (Biliary Hepatitis):** At autopsy in 36 cases of extrahepatic biliary obstruction of various causes the basophilic bodies of the liver cells were only somewhat decreased or not at all. This reduction of basophilia was less obvious in comparison with the degree of liver cell damage as indicated by other morphologic criteria than in the previously described conditions. The liver cells which were definitely imbibed with bile pigment or which contained bile pigment granules revealed a significant decrease in basophilic substance, but the other liver cells showed even in routine hematoxylin-eosin sections a dark blue hue. Liver cell damage and reduced basophilia were seen mainly in the central zone. The actively proliferated Kupffer cells, the cytoplasm of which was loaded with bile pigment, were almost free of basophilic bodies. In the portal triads no or only few basophilic body-containing histiocytes were noted. If the segmented granulocytes which had densely infiltrated the portal triads indicated a secondary infection (acute perilymphangitis) the basophilic body-containing Kupffer cells and histiocytes in the portal triads were significantly increased in this condition. Biopsy specimens of 34 patients with biliary hepatitis and 11 with infected biliary hepatitis (extrahepatic obstruction with acute perilymphangitis) exhibited similar findings.

**Cirrhosis:** In 86 necropsy specimens of Laennec's cirrhosis, the basophilic bodies varied considerably in amount and distribution. In a predominantly fatty form with a moderate degree of reconstruction the cytoplasmic basophilia was, as a rule, significantly decreased throughout the lobule. The cytoplasmic rims around the smaller or larger fat vacuoles revealed little basophilia. The small nuclei possessed small and indistinct nucleoli. The variations in the distribution of basophilic bodies were more outstanding in other forms of cirrhosis and varied from nodule to nodule. The liver cells of some of the regenerated nodules were uniformly rich in basophilic bodies and possessed large nuclei and nucleoli. In the active, progressive type of cirrhosis the liver cells in the centers of many of the nodules were necrotic, their cytoplasm devoid of basophilic bodies. The remaining liver cells of these nodules contained small nuclei and small indistinct nucleoli; other nodules were composed of relatively large cells with moderately large nuclei and large nucleoli with few basophilic bodies in the perinuclear zone (fig. 3A). Central eosinophilic areas without frank necrosis were rather common. In addition to the zonal reduction of basophilic bodies, scattered foci of liver cells appeared completely free of basophilia. Decrease of hepatocellular basophilic bodies was especially notable in patients with severe jaundice. The Kupffer cells were frequently increased in number and size, and their enlarged cytoplasm was rich in basophilic bodies. Also in the trabeculae the cellular infiltrates contained histiocytes rich in cytoplasmic basophilia.

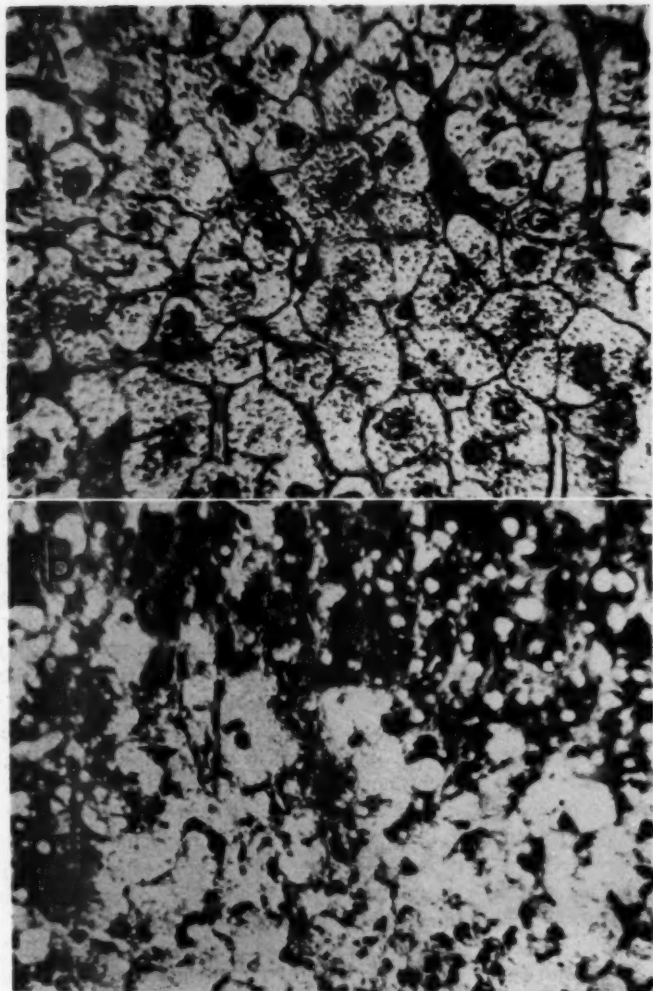


Fig. 3.—*A*, Laennec's cirrhosis. The nuclei and the nucleoli are large. The basophilic substance is decreased in the liver cells. There is some accumulation of basophilic bodies around the nuclei (methyl green-pyronine;  $\times 400$ ).

*B*, rat liver 24 hours after administration of 0.03 Gm. of carbon tetrachloride. In the central zone the anuclear cells and cell fragments are free of basophilic bodies. The liver cells of the intermediate zone show some reduction of basophilic material (methyl green-pyronine;  $\times 400$ ).

In the cases of so-called compensated or inactive cirrhosis the cytoplasm of the liver cells contained, as a rule, normal amounts of basophilic bodies. Some nodules showed, however, an increase of the cytoplasmic basophilia and large nuclei and nucleoli. Cytoplasmic basophilia of the mesenchymal cells (Kupffer cells and periportal histiocytes) was usually increased. There was no significant difference between postnecrotic and Laennec's cirrhosis in the type of the distribution of the basophilic bodies.

In 75 biopsy specimens from 63 patients with cirrhosis similar alterations in the distribution of the basophilic material were noted. There was a fairly good parallelism between the reduction of the basophilic bodies in the liver cells, on one side, and liver cell damage and clinical activity, on the other.

*Variation of Basophilic Body Distribution in Experimental Hepatic Injury.—*

The basophilic bodies were absent from the necrotic cells and cell fragments of the centrolobular portion in rats (fig. 3B) or mice intoxicated with carbon tetrachloride and from the peripheral zone in rats which had received allylformate. The cytoplasm of the cells free of basophilic bodies was brightly eosinophilic. In mice the basophilic bodies disappeared from the cells of the central zone before fatty metamorphosis developed to any significant degree after intraperitoneal injection of carbon tetrachloride. Kupffer cells in the necrotic zone were often rich in basophilic bodies. The liver cells in the vicinity of the necrotic or fatty zone revealed less basophilia than normal, though other changes could not be made out. In carbon tetrachloride intoxication the peripheral zones revealed some increase in basophilic bodies, due to regenerative changes.

COMMENT

The study of the cytoplasmic basophilia (as an index of pentosenucleic acid distribution in the liver) is of academic and diagnostic interest. It may facilitate the effort to correlate alterations of the epithelial or mesenchymal cells with functional disturbances, especially concerning the protein metabolism and, in the case of the epithelial cells, serum albumin formation. The increased basophilia of the hepatic cells in brown atrophy or in various forms of compression atrophy indicates intact function of these cells. The increase is probably more apparent than real; the cells appear much smaller, and the basophilic bodies may, therefore, be more concentrated. It appears that in such atrophy other constituents disappear before the basophilic bodies do. Similarly, moderate deposition of lipofuscin and hemosiderin pigments does not influence the basophilic body content of the liver cells. Bile pigment deposition is not necessarily associated with a decrease of basophilic bodies even if other signs of liver cell damage are noticeable. The basophilic bodies are absent only from cells in which diffuse imbibition of bile pigment suggests a damage of the cells. In such conditions the serum albumin level is not necessarily reduced; this is also in keeping with intact formation of serum protein by the liver cells.

Fatty metamorphosis, at least in its moderate degree, is not necessarily associated with reduced functional ability (or disturbance of protein formation) as indicated from the presence of basophilic bodies. However, in the more advanced degrees of fatty liver the basophilic bodies are almost completely absent. In some

types of cytoplasmic disorganization, as in hydropic changes or in various forms of coagulation ("hyaline") necrosis, the basophilic bodies are absent. The pronounced acidophilic (eosinophilic) character of the cytoplasm of hepatic cells in coagulation necrosis can be explained by the combination of two factors: (1) basophilic body depletion of the liver cells and (2) the denaturated cytoplasmic protein's increased absorption of the acid dyes.<sup>20</sup> The basophilic body depletion of hepatic cells in which the nuclei are preserved could be called acidophilic (eosinophilic) degeneration; the basophilic body depletion combined with severe nuclear changes (pyknosis, karyorrhexis, karyolysis), acidophilic (eosinophilic) necrosis. The functional significance of the large basophilic bodies appearing in the course of infections<sup>21</sup> is at present not clearly understood.

A functional relationship of nucleoli and cytoplasmic pentose nucleoprotein has been considered in that the cytoplasmic nucleic acid is supposed to be under the control of the nucleolus and the latter in turn under that of the nuclear chromatin.<sup>22</sup> In animals starved or restricted to a low protein diet a decrease in the size and volume of the nucleolus proceeded simultaneously with a decrease or disappearance of the cytoplasmic basophilia.<sup>15</sup> However, enlargement of the nucleoli and limitation of the cytoplasmic basophilia to the perinuclear zone were observed in rats kept on a protein-free diet for a long period.<sup>23</sup> In this study the nucleoli appeared large in regenerating liver cells containing increased amounts of cytoplasmic pentose nucleic acid. In the liver cells with partial or complete depletion of the cytoplasmic pentose nucleic acid the nucleoli were either small, just as the nuclei were, or large in moderately enlarged nuclei. The latter type reveals usually some accumulation of basophilic bodies in the perinuclear zone. The enlargement of the nucleoli of the depleted hepatic cells may be interpreted as a compensatory mechanism; the nucleolus, an organelle of the pentose nucleic acid synthesis, tries to increase the production of basophilic bodies.

The increase or appearance of basophilic bodies in Kupffer cells and in histiocytes in the portal triads, e. g., in viral hepatitis or active cirrhosis, requires an explanation. Phagocytosis of basophilic material released by disintegrating epithelial cells is not probable since the basophilia of the hepatic cells disappears as soon as the epithelial cells become damaged. It is an obvious sign of a hepatic mesenchymal reaction which may be a primary process in these diseases or a response to the damage to the hepatic cells. The appearance of the basophilic bodies in Kupffer cells and histiocytes can be considered a morphologic sign of the formation of serum gamma globulin (of which a small fraction may be antibody globulin), which these cells as a part of the reticuloendothelial system should be able to synthesize. In these conditions the serum gamma globulin level is, as a rule, elevated.<sup>24</sup>

The study of the distribution of basophilic bodies in the liver may also assist in the histological differential diagnosis of various hepatic disorders. For instance, toxic hepatitis—human, as well as experimental—shows a zonal decrease or a

20. Williams, W. L., and Frantz, M.: *Anat. Rec.* **100**:547, 1948.

21. Rich, A. R., and Berthrong, M.: *Bull. Johns Hopkins Hosp.* **85**:327, 1949.

22. Thorell, B.: *Acta med. Scandinav.* **117**:334, 1944.

23. Stowell, R. E.: *Cancer* **2**:121, 1949.

24. Popper, H.; de la Huerga, J.; Steigmann, F., and Slodki, M.: *J. Lab. & Clin. Med.* **35**:391, 1950.

complete disappearance of the basophilic bodies with resulting acidophilia of the involved zones. The depletion of basophilic bodies is usually centrilobular but it may involve the midzone and extend to the peripheral zone if the injury is sufficiently severe. In contrast to this the basophilic body-depleted cells are scattered irregularly throughout the lobule in viral hepatitis. In addition, in viral hepatitis there is an impressive mesenchymal reaction, characterized by mobilization of the Kupffer cells and numerous histiocytes in the portal triads,<sup>23</sup> both rich in basophilic bodies. Persistence of the viral hepatitis, even after the liver cell damage and most of the clinical findings have subsided, may be indicated by the basophilia of the mesenchymal elements in the portal triads. In toxic hepatitis, at least in human material, the mesenchymal reaction is less pronounced or completely absent.<sup>25</sup> In extrahepatic biliary obstruction the Kupffer cells are mobilized, but they are laden with bile pigment and do not contain basophilic bodies. It is interesting that in this condition the serum gamma globulins are hardly elevated.<sup>24</sup> This is in contrast to infected extrahepatic obstruction, in which numerous basophilic body-containing histiocytes are found in the portal triads and many of the Kupffer cells contain basophilic bodies besides bile pigments.

In cirrhosis the basophilia seems to mirror the activity of the disease process. Depletion of the hepatocellular basophilic bodies, usually associated with high numbers of basophilic body-containing Kupffer cells and histiocytes in the trabeculae and portal triads, characterizes, clinically as well as biochemically, cases in which the disease is active, especially if the presence of jaundice indicates such activity. The basophilic body-depleted liver cells possess either small nucleoli in small nuclei or large nucleoli in moderately enlarged nuclei as has been recently pointed out.<sup>3</sup> Variation of the basophilic body distribution throughout the liver as seen in cirrhosis suggests variation of the functional state in different lobules and nodules. The actively regenerating areas reveal the morphological signs of hyperfunctioning cells. In arrested cases of cirrhosis the basophilic body content of the liver cells does not differ from the normal and the mesenchymal cells (Kupffer cells and portal histiocytes) do not show cytoplasmic basophilia.

#### SUMMARY

By various staining methods combined with specific enzymatic digestion, the cytoplasmic basophilia of the hepatic and mesenchymal cells was studied in specimens of liver obtained from humans at autopsy and by biopsy and from experimental animals. In general, the damaged liver cells show a marked decrease, while mobilized Kupffer cells and proliferated histiocytes in the portal triads reveal an increase, of the cytoplasmic basophilia.

In moderate fatty metamorphosis and pigment deposition the cytoplasmic basophilia is not decreased. In the various types of hepatic atrophy the basophilic bodies appear increased because of the higher concentration in a smaller cell volume. In extensive fatty metamorphosis, hydropic degeneration and partial or complete coagulation necrosis the basophilic bodies are absent and the cytoplasm is brightly eosinophilic. Regenerating liver cells and proliferating small bile ducts are rich in cytoplasmic basophilia.

25. Popper, H., and Franklin, M.: Viral Versus Toxic Hepatic Necrosis, *Arch. Path.* 46:338 (Oct.) 1948.

In viral hepatitis the mesenchymal cells are rich in basophilic substances while the epithelial cells depleted of basophilic bodies are scattered irregularly throughout the lobule. In the fulminant form of viral hepatitis almost all the liver cells are depleted of basophilic bodies. In toxic hepatitis the depletion is uniform in the central zone and the mesenchymal reaction is minimal. In obstructive jaundice the depletion of the basophilic substance of the liver cells is slight despite the often severe liver cell damage. In cirrhosis the decrease of basophilic bodies in the liver cells and the increase in the mesenchymal cells mirror the activity of the disease process. The picture becomes even more variegated since the epithelial cells of regenerating nodules may be very rich in basophilic material.

The alterations of the distribution of cytoplasmic basophilia (regarded as an index of pentose nucleic acid) may offer a histological means by which to judge the functional state of hepatic epithelial and mesenchymal cells, since pentose nucleic acid plays an important role in protein formation and the latter is one of the main functions of these cells. In addition, the characteristic alterations of the distribution of the basophilic substance offer criteria for the histological differential diagnosis of hepatic diseases.

## MORPHOLOGIC LESIONS DUE TO ACUTE AND SUBACUTE POISONING WITH ANTIFREEZE (ETHYLENE GLYCOL)

DAVID E. SMITH, M.D.

ST. LOUIS

REPORTS of fatal cases and experimental studies of poisoning by ethylene glycol have dealt, for the most part, with instances in which death either occurred very shortly after poisoning or was delayed for months. This report describes observations made at autopsy on two patients who survived about 12 days after ingestion of a "permanent-type antifreeze" solution and whose tissues, particularly the liver and the kidney, contained lesions which are distinctly different from those that occurred in 6 cases of acute poisoning by the same substance. The lesions resulting from this type of poisoning are to a certain extent specific, and their recognition is important, because the widespread and increasing use of "permanent-type antifreeze" solutions in the radiators of internal combustion engines and the lack of general knowledge of the dangers of consuming such fluid have resulted in numerous deaths during the past decade.

Commercial antifreeze solutions are composed essentially of ethylene glycol ( $\text{HO}-\text{CH}_2-\text{CH}_2-\text{OH}$ ), but preparations are now being marketed that are composed of propylene glycol ( $\text{HO}-\text{CH}_2-\text{CHOH}-\text{CH}_3$ ), and Wordley<sup>1</sup> stated that some contain diethylene glycol ( $\text{HO}-\text{CH}_2-\text{CH}_2-\text{O}-\text{CH}_2-\text{CH}_2-\text{OH}$ ). The latter compound is familiar as the fatal vehicle in the poisonings due to an "elixir of sulfanilamide" which occurred in the United States in 1937.<sup>2</sup> Propylene glycol is much less toxic than the other two substances and is consequently less likely to be the cause of fatal poisoning.<sup>3</sup>

The literature of this subject, particularly as it pertains to ethylene glycol, has been recently reviewed by Pons and Custer,<sup>4</sup> Widman,<sup>5</sup> Milles<sup>6</sup> and Hagemann and Chiffelle.<sup>7</sup> In most cases to which these authors refer the poisonous fluid was imbibed with intention of producing inebriation, but poisoning has resulted from the

The material reviewed in this report is from the files of the Armed Forces Institute of Pathology, Washington 25, D. C.

From the Fourth Medical Laboratory, European Command, United States Army, and the Department of Pathology, Washington University School of Medicine.

1. Wordley, E.: *J. Clin. Path.* **1**:44, 1947.

2. Geiling, E. M. K., and Cannon, P. R.: *Pathologic Effects of Elixir of Sulfanilamide (Diethylene Glycol) Poisoning: A Clinical and Experimental Correlation; Final Report*, *J. A. M. A.* **111**:919 (Sept. 3) 1938.

3. Laug, E. P.; Calvery, H. O.; Morris, H. J., and Woodard, G.: *J. Indust. Hyg. & Toxicol.* **21**:173, 1939.

4. Pons, C. A., and Custer, R. P.: *Am. J. M. Sc.* **211**:544, 1946.

5. Widman, C.: *Acta med. Scandinav.* **126**:295, 1946.

6. Milles, G.: *Ethylene Glycol Poisoning, with Suggestions for Its Treatment as Oxalate Poisoning*, *Arch. Path.* **41**:631 (June) 1946.

7. Hagemann, P. O., and Chiffelle, T. R.: *J. Lab. & Clin. Med.* **33**:573, 1948.

use of the essential chemical as a vehicle in topical medicaments.\* Hagemann and Chiffelle<sup>7</sup> have summarized the clinical events as: coma, acidosis, hypertension, convulsions, leukocytosis, albuminuria, microhematuria, nitrogen retention, lowered carbon dioxide combining power of the serum, and increase of protein and cells in the cerebrospinal fluid. Nonfatal cases have presented similar but less severe features.

The presence of doubly refractile crystals of calcium oxalate in the renal tubules of animals poisoned with ethylene glycol was observed by Mayer<sup>9</sup> as early as 1903 and is a constant feature of poisoning with that substance. Pons and Custer,<sup>4</sup> in reviewing material from 18 fatal human cases, observed similar crystals in the brain and leptomeninges which, combined with distinct meningitis and evidence of cellular damage in the brain, were interpreted as the principal lesion responsible for death. They reported that the renal tubules did not contain significantly damaged epithelium, despite the presence of large numbers of crystals, and that in rapidly fatal cases the clinical symptoms were more suggestive of damage to the central nervous system than renal failure. Hagemann and Chiffelle<sup>7</sup> more recently suggested that the disturbed metabolic status of poisoned individuals be attributed to diffuse damage of the capillaries, which was indicated by the presence of scattered microscopic parenchymal hemorrhages.

#### REPORT OF CASES OF SUBACUTE POISONING

CASE 1 (A. F. I. P. acc. no. 203,707).—A white man aged 21 years consumed about half a canteen-cupful (250 cc.?) of a beverage made by mixing "permanent-type antifreeze" solution with water. Symptoms did not develop immediately, but at the time the patient was admitted to the hospital, 22 hours after consuming the beverage, he had experienced nausea, vomiting, pain in the eyes, headaches and abdominal pain. He was lethargic and confused but responded well to stimuli. Temperature, blood pressure, pulse rate and respiratory rate were normal. After the first day his temperature was 99 to 101 F. He continued to be lethargic, complained of blurred vision and gradually became anuric. The nonprotein nitrogen of the blood rose to 160 mg. per hundred cubic centimeters terminally on the twelfth day; the highest blood pressure was 167 systolic and 118 diastolic on the ninth day. Symptoms of his terminal course suggested uremia with pulmonary edema and respiratory failure.

CASE 2 (A. F. I. P. acc. no. 203,700).—A white man aged 20 years had been drinking "potato schnapps" of uncertain quality indiscriminately for 10 weeks. During the four days prior to admission he had intermittent attacks of abdominal pain, vomiting and hiccup, followed by a burning sensation in the epigastrium. Vomiting occurred immediately after the ingestion of all foods and liquids. A severe cough productive of sputum began at the same time, and on the day of admission he had one chill. His temperature was 99.6 F., but his blood pressure, pulse rate, and respiratory rate were normal. There was tenderness in the epigastrium, the right upper quadrant of the abdomen and bilaterally over the costovertebral angles. The deep reflexes were hyperactive bilaterally. During hospitalization the patient was anuric except for a small quantity of bloody urine removed by catheterization on the day of death; his temperature became normal and remained so, and the respiratory rate declined during the last few days. His course was suggestive of progressive uremia and acidosis, and he died in convulsions on the eleventh day after the onset of symptoms.<sup>10</sup> The nonprotein nitrogen of the blood rose to 399 mg. per hundred cubic centimeters and the carbon dioxide combining power to 18.3 cc. per hundred cubic centimeters on the day of death. The plasma chlorides were 264 mg. per hundred cubic centimeters on the day after admission, rose to 330 mg. on the fifth day and fell to 281 mg. terminally.

8. Doerr, W.; Kraft, A., and Rauschke, J.: *Klin. Wchnschr.* **24/25**:749, 1947.

9. Mayer, P.: *Ztschr. f. physiol. Chem.* **38**:135, 1903.

10. The autopsy was performed by Capt. Robert J. Brimi, Medical Corps, Army of the United States.

*Postmortem Examinations.*—Ascites was present in both case 1 and case 2 (400 cc. and 1,500 cc., respectively). In both cases the pleural cavities each contained about 400 cc. of clear fluid. In case 1 edema of the interstitial tissue was observed throughout the body and was especially prominent beneath the pectoral muscles and retroperitoneally. Advanced congestion and edema of the lungs were present in both cases. The livers were enlarged (2,250 Gm. and 3,150 Gm.), light tan and slightly friable. The cut surfaces had slightly accentuated fine gray lobular markings, and there were numerous yellow foci about 0.5 mm. in diameter. Each kidney (fig. 1) was enlarged to 300 Gm. or more. The capsules were stripped with difficulty, to expose smooth yellow surfaces showing mottled red areas 4 to 8 cm. in diameter. These areas were neither raised nor depressed and were of grossly irregular outline. In the central portions of the larger ones there were irregular foci of grayish yellow, necrotic tissue, 1 or 2 cm. in diameter. On incision the renal substance bulged, the cortex was thickened, and the pars convoluta was yellow and prominent. The large red foci on the surface extended through about half the thickness of the cortex and never involved the medulla. The renal pelvic mucosa was thickened and contained scattered petechiae. In case 1 the brain was remarkable only for a moderate prominence of the cerebellar tonsils and a moist, mottled appearance of the cut surfaces of the basilar gray masses. In case 2 it was described as normal. Scattered petechiae were present in various organs. Except for congestion of the mucosa of the stomach, the cecum and the ascending colon with edema and slight ulceration over prominent lymphoid follicles of the colon in case 2, there were no other significant gross pathologic changes.

*Microscopic Examinations.*—In sections of the liver, in each case, there were, around or adjacent to central veins, many foci of several hundred cells with pyknotic nuclei and advanced hydropic degeneration of the cytoplasm (fig. 2A). At the edges the hydropic cells were continuous through transitional forms with the cells in the hepatic cords (fig. 2B). In properly fixed and stained sections neither fat nor glycogen was stained in the cytoplasm of these cells. In case 1 the portal triads were slightly enlarged, but there was no other evidence of cirrhosis. With polarized light (fig. 3) many small doubly refractile crystals were apparent in the nuclei of cells in otherwise unaffected portions of the hepatic parenchyma. This finding was not constant or uniform in the sections, but when it occurred it was present throughout several low power fields. In case 2 a few similar intranuclear crystals were present only in the liver.

In the kidneys (fig. 4) the epithelium of many tubules in the cortex was composed of flat, dark cells. In some proximal convoluted tubules the cells had undergone extensive hydropic degeneration; in other tubules the epithelium was totally destroyed. In the lumens of the tubules there were many sheaf-shaped clear crystals that were brilliantly doubly refractile under polarized light. Some crystals were surrounded by large multinucleated cells. There were also generalized interstitial edema and a few scattered lymphocytes. The glomeruli, in general, were not remarkable, but in case 1 some contained a few adhesions and evidence of slight proliferation of the epithelium. Sections of the red lesions in the cortex had the appearance of infarcts. Within the lesions the vessels contained no significant changes, but in a section from an adjacent block there was necrosis of the outer half of the wall in several medium-sized arteries, associated with heavy cellular infiltration. With polarized light a few small crystals could be seen in the nuclei of occasional cells in glomeruli and tubules in case 1, and in case 2 some crystals lay in the interstitial tissue outside the tubules.

In the lungs there were small foci of bronchopneumonia, large amounts of granular precipitated protein in the alveoli, advanced congestion of the vascular bed and subacute bronchitis. In case 1 the heart was not remarkable, but in case 2 there were foci of interstitial collections of mononuclear cells and lymphocytes in the myocardium and beneath the epicardium. In the myocardium there were occasional Anitschkow myocytes about blood vessels but no damaged muscle fibers, and beneath the epicardium a few eosinophilic granulocytes were seen.

In sections of the brain, small doubly refractile crystals were present in the walls of blood vessels (fig. 5) and occasionally in the substance in case 1. In the cerebellum some folia were without Purkinje cells, and in some well fixed portions the Purkinje cells were shrunken and pyknotic. No satisfactory sections of the brain were available in case 2.

In sections of the other organs in case 1 intranuclear doubly refractile crystals were present in splenocytes and fibroblasts in the spleen, interstitial fibroblasts in the lungs, epithelial cells and interstitial fibroblasts in the pancreas, lymphocytes in lymph nodes, epithelial cells in

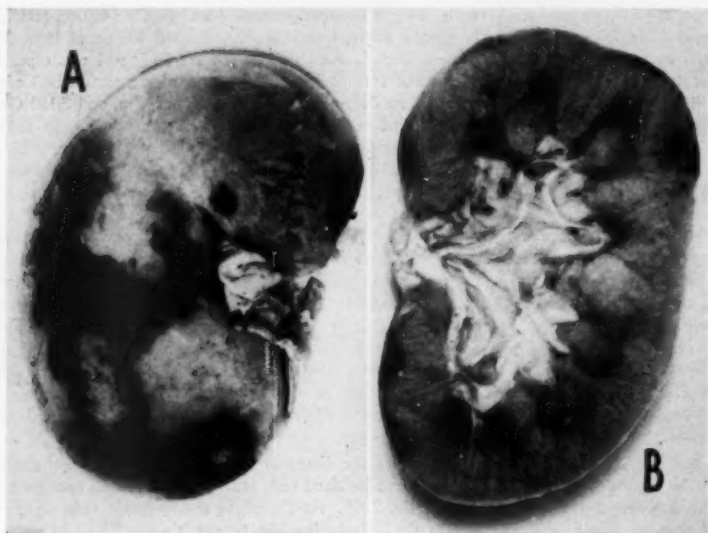


Fig. 1 (case 1).—A kidney from a young man suffering from subacute poisoning by ethylene glycol;  $\times \frac{1}{2}$ . *A*, exterior; *B*, interior. The large, irregular, red lesions had central foci of necrosis and extended through half the thickness of the cortex.

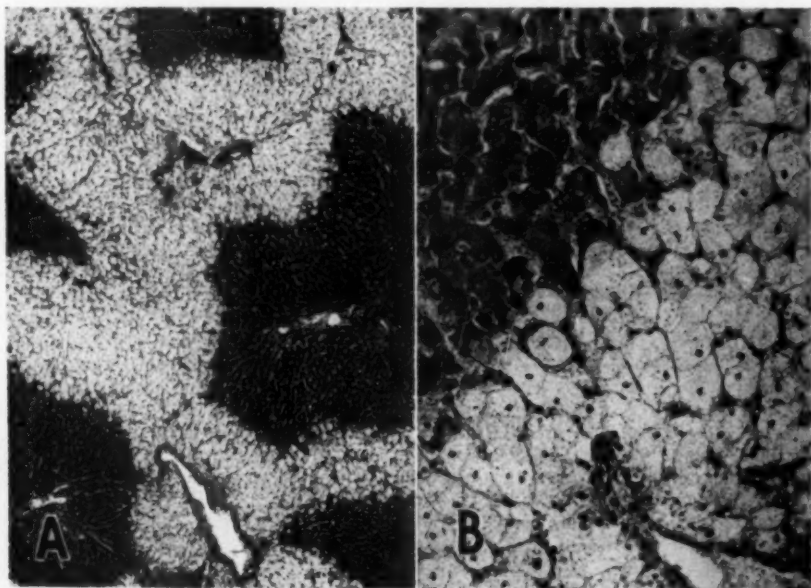


Fig. 2 (case 2).—*A*, central hydropic degeneration of the cells of the hepatic lobules; hematoxylin and eosin stain;  $\times 33$ . *B*, edge of a zone of hydropic cells in the liver; hematoxylin and eosin stain;  $\times 288$ .

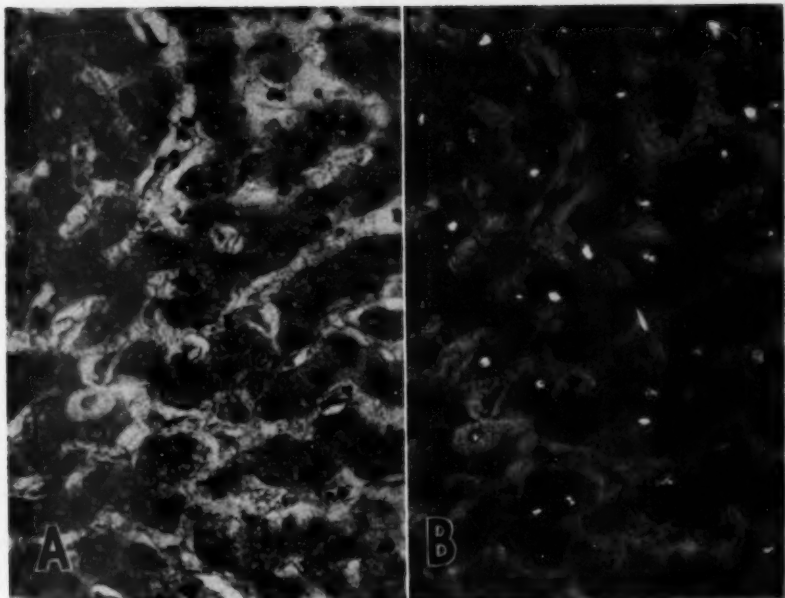


Fig. 3 (case 1).—An intranuclear doubly refractile substance in the hepatic cells and Kupffer cells; hematoxylin and eosin stain;  $\times 480$ . *A* shows a field taken with the polarizer and analyzer uncrossed, and *B* was the same field taken with the polarizer and analyzer crossed. There was no other lesion in this field.

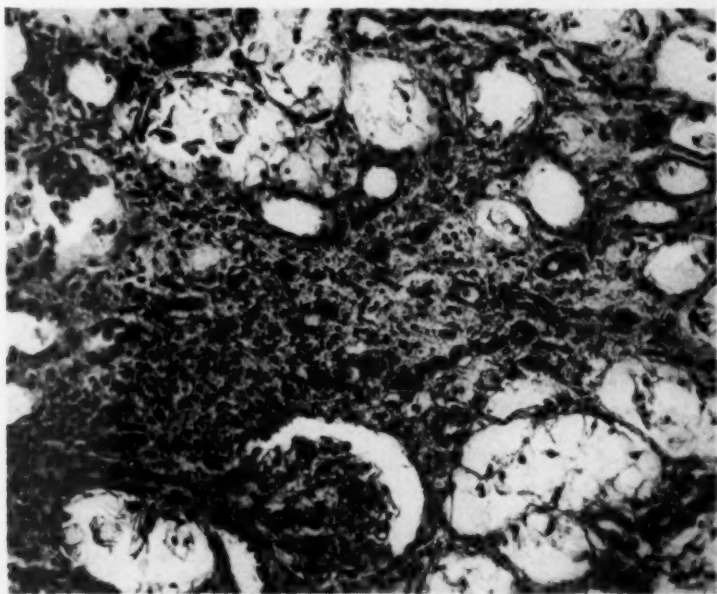


Fig. 4 (case 1).—Low columnar epithelium in distal tubules, hydropic epithelium in proximal convoluted tubules, crystals, and a focus of interstitial inflammation in the kidney; hematoxylin and eosin stain;  $\times 185$ .

the prostate, fibroblasts in the interstitial tissue of the testis and cortical cells in the adrenal. There were also in case 1 minimal subacute interstitial pancreatitis, slight cellular interstitial infiltration of the diaphragm, necrosis of follicles in some lymph nodes and small focal hemorrhages in the submucosa of the stomach.

*Comment on Cases of Subacute Poisoning.*—Hydropic changes such as those present in the central portions of the hepatic lobules in these two cases of subacute poisoning have not been previously emphasized in connection with poisoning by ethylene glycol but are identical with the lesions illustrated in reports of poisoning by diethylene glycol,<sup>2</sup> dioxane,<sup>11</sup> and carbon tetrachloride.<sup>12</sup> Doerr<sup>13</sup> referred to observations made by Hildebrand in a case in which, he stated, there



Fig. 5 (case 1).—Interstitial doubly refractile crystals in the wall of a blood vessel of the cerebrum photographed with polarized light; hematoxylin and eosin stain;  $\times 185$ .

was hydropic vacuolar epithelial degeneration in the liver of a patient who lived 17 days after poisoning. It is significant that this type of change has occurred in the tissues of patients who have survived poisoning with one or the other of a small group of organic substances for a period of about two weeks. It has not been recognized in poisoning by ethylene glycol, probably because most of the patients reported have died within 24 to 48 hours and the various series of animal experiments have been designed either to cause death in a very short length of time or to have the animals survive for a period of months.

11. Barber, H.: *Guy's Hosp. Rep.* **84**:267, 1934.

12. Andrews, W. H. H., and Macgraith, B. G.: *Ann. Trop. Med.* **42**:95, 1948.

13. Doerr, W.: *Virchows Arch. f. path. Anat.* **313**:137, 1944.

In case 1, as well as in cases 3, 4, 5, 6 and 7 of acute poisoning that occurred at the same time, a small amount of the fluid imbibed was obtained, and qualitative chemical analysis indicated that it contained ethylene glycol but no methyl alcohol. No further determinations of the exact composition of this fluid were made. Particularly, it was not determined by analysis that it was free of other substances, such as diethylene glycol. The possibility that it might have contained other substances known to cause the hydropic lesion in the liver is considered remote for several reasons. First, these two cases occurred separately; second, it was reasonably certain that the poison, particularly in case 1, was the usual commercial ethylene glycol "antifreeze"; third, typical lesions of acute poisoning were present in the organs of five other patients poisoned with the same fluid imbibed by the patient in case 1; fourth, Hildebrand's observations<sup>14</sup> are suggestively supportive, and fifth, similar survival periods have not been reported in other cases or experiments.

The necrotic foci in the renal cortices have also been previously mentioned only in Doerr's<sup>13</sup> comments on Hildebrand's observations in two cases in which the patients survived eight days after poisoning. They are, however, very similar to lesions in the patients who were poisoned by diethylene glycol<sup>2</sup> used as a vehicle for sulfanilamide, and possibly like the lesions observed by Barber<sup>11</sup> in cases of poisoning by dioxane. The changes in the viable tubular epithelium in these cases are particularly significant, as they indicated extensive toxic damage of those structures. These cases as well as those of Doerr,<sup>13</sup> and the experimental observations of Laug and associates,<sup>8</sup> Morris and associates<sup>15</sup> and Hanzlik and associates<sup>16</sup> indicate that actual renal damage can be an important effect of the poison. Like the hepatic lesion, the hydropic and degenerative lesion of the kidney is a well recognized feature of poisoning by substances related to ethylene glycol when the subject has survived for longer than 48 hours.<sup>17</sup>

The slight diffuse interstitial inflammation observed in various sites, including the heart, the pancreas and the diaphragm, is another feature not previously emphasized as part of the morphologic changes in poisoning by ethylene glycol. The generalized hydrops in these two cases is also worthy of mention, although it is interpreted as a phenomenon secondary to the changes in the kidneys and the circulatory system.

Doubly refractile crystals, presumably of calcium oxalate, were present within the renal tubules, in various interstitial sites and about blood vessels in the leptomeninges, as has been described by other authors.<sup>4</sup> Particularly noteworthy, however, was the occurrence of a doubly refractile substance within the nuclei of various cells in many organs not only in the two cases of subacute poisoning but in several of the cases of acute poisoning described in this report. This finding is presented with frank distrust that it is more than an interesting artefact. All the slides used in this study were cut and stained in the same laboratory during a relatively short period of time. The intranuclear doubly refractile substance was present in some slides of each of two sets prepared from the original blocks. The formaldehyde

14. Hildebrand, cited by Doerr.<sup>13</sup>

15. Morris, H. J.; Nelson, A. A., and Calvery, H. D.: *J. Pharmacol. & Exper. Therap.* **74**:266, 1942.

16. Hanzlik, P. J.; Lawrence, W. S., and Laqueur, G. L.: *J. Indust. Hyg. & Toxicol.* **29**:233, 1947.

17. Geiling and Cannon.<sup>2</sup> Barber.<sup>11</sup>

fixation and even the embedding of tissue from the various persons poisoned occurred at widely separated places and times. In several of the cases material was embedded after it had been fixed and stored in formaldehyde solution U. S. P. for almost two years, and only very rare and questionable examples of the phenomenon were present in the slides prepared. The intranuclear doubly refractile substance in a section of adrenal in case 1 was insoluble in normal potassium hydroxide and soluble in normal hydrochloric acid. Crystals of calcium oxalate react in the same manner to these reagents, and the test is presumptive, although not conclusive, for that substance. The solubility of the crystals in acid solutions was possibly responsible for their absence in the tissues that had been stored in formaldehyde solution. Attempts to reproduce the intranuclear crystals as well as the other lesions of subacute poisoning by administering ethylene glycol to white mice have been unsuccessful.

#### REPORT OF CASES OF ACUTE POISONING

CASES 3,<sup>18</sup> 4, 5, 6 and 7 (A. F. I. P. acc. nos. 203,702; 203,703; 203,704; 203,705; 203,706).—The patients were 28, 30, 25, 19 and 21 years of age, respectively, and were all white soldiers who partook of an unknown amount of a beverage made by mixing "antifreeze" solution and water. Within eight hours after consumption of the fluid, vomiting, increasing stupor, unconsciousness and respiratory failure began to develop. Death occurred in the respective patients 18, 20, 26, 26 and 32 hours after they drank the fluid.

*Postmortem Examinations.*—Gross changes in the viscera consisted of congestion and edema of the lungs, congestion of the viscera and petechiae on various mucous and serous surfaces. Bilateral hydrothorax of 100 to 200 cc. of fluid was present in cases 5, 6 and 7. Prominence of the cerebellar tonsils was observed in cases 4 and 7, and the cut surfaces of all the brains were abnormally moist.

*Microscopic Examinations.*—In sections of the heart in cases 5 and 7 there was distinct interstitial myocarditis, and in case 6 a few polymorphonuclear granulocytes, as well as focal hemorrhages, were present in the epicardium. Slight bronchopneumonia and advanced congestion and edema were observed in the lungs in all cases, and pronounced bronchitis and tracheitis in cases 4 and 7. Changes in the livers, present in only two cases, consisted of foci of a few cells with partially hydropic cytoplasm in case 3 and slight enlargement and cellular infiltration of the portal triads in case 5. In the kidneys there were large numbers of sheaflike doubly refractile crystals in the tubules but no evidence of reaction on the part of the tissue (fig. 6). The crystals were principally in the proximal convoluted tubules, but in the case with the longest survival they were equally distributed in the proximal and distal convoluted tubules and the collecting tubules. In sections of the brain there were mononuclear cells and a few polymorphonuclear granulocytes in the meninges in cases 4, 5, 6 and 7; a slight perivascular infiltrate of lymphocytes in all cases, and microscopic hemorrhages in case 3. Suggestive changes in the neurons had occurred in all cases but were considered equivocal because of inadequate fixation. Doubly refractile crystals about the blood vessels and occasionally in the substance of the brain were present in all cases. A distinct leukocytic infiltration of the muscularis and submucosa of the stomach was present in cases 4, 5 and 7, and there was slight esophagitis in cases 3, 4 and 5. Evidence of slight interstitial inflammation was observed in some other tissues such as pancreas, lymph nodes, prostate, testis and diaphragm in various cases. Cells in which intranuclear doubly refractile crystals were present included the hepatic cells in cases 3 and 7, leukocytes and cells of the alveolar walls in foci of bronchopneumonia in cases 6 and 7, splenocytes in cases 6 and 7, interstitial fibroblasts and epithelial cells of the acini in the pancreas in cases 6 and 7, epithelial cells of the prostate in case 6 and interstitial fibroblasts in the testis and cortical cells in the adrenal in case 7.

18. The autopsy was performed by Capt. John J. Donovan, Medical Corps, Army of the United States.

CASE 8<sup>19</sup> (A. F. I. P. acc. no. 203,701).—A white man 31 years of age was found dead in bed. Some comrades said that he had been drinking heavily for several days before and that he had drunk some "antifreeze" solution.

*Postmortem Examination.*—Necropsy was not performed until 24 hours after discovery of the body and an unknown time after death. The tissues were badly autolyzed. Gross pathological changes consisted of advanced congestion and edema of the lungs (2,100 Gm.) and congestion of all the viscera.

Toxicological analysis of blood, urine and gastric contents revealed no significant evidence of ethyl or methyl alcohol and no metallic, phenolic or aniline poisons.

*Microscopic Examination.*—The tissues were so autolyzed that recognition of pathological changes was impossible. The significant findings were the presence of doubly refractile crystals in the renal tubules and about the blood vessels of the brain.

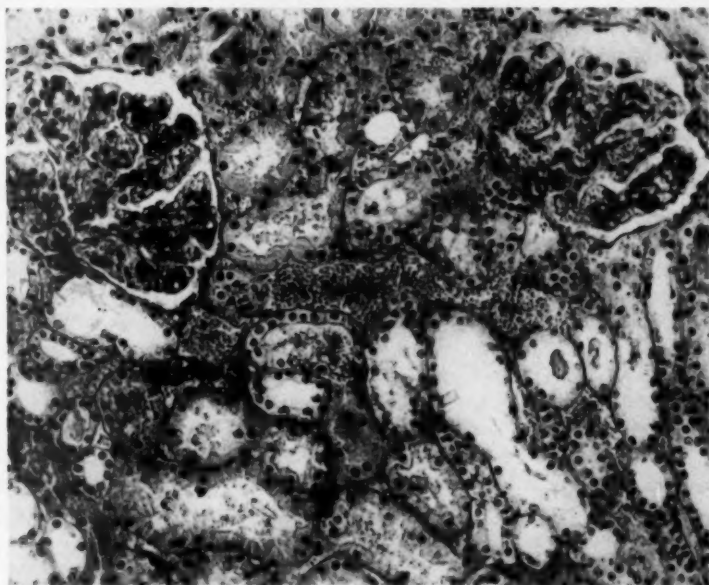


Fig. 6 (case 3).—Kidney illustrating acute ethylene glycol poisoning; hematoxylin and eosin stain;  $\times 200$ . Crystals of calcium oxalate were present within the convoluted tubules without a significant reaction in the epithelium.

*Comment on Cases of Acute Poisoning.*—Most of the lesions observed in these cases of acute poisoning are the same as those described previously in various reports.<sup>4</sup> The slight interstitial inflammation in the heart, the stomach and various organs was similar to that in the cases of subacute poisoning and is a significantly constant feature. Holck<sup>20</sup> reported a slight degree of myocarditis in animals, but no other report of a similar lesion could be found. The intranuclear doubly refractile matter was observed in tissues in these cases as well as in those in cases 1 and 2, but nothing more could be concluded as to their significance.

19. The autopsy was performed by Capt. Gerald E. Cosgrove, Medical Corps, Army of the United States.

20. Holck, H. G. O.: Glycerin, Ethylene Glycol, Propylene Glycol and Diethylene Glycol: Report on Feeding Experiments with Rats, J. A. M. A. **109**:1517 (Nov. 6) 1937.

Case 8 is an example of the persistence of the characteristic doubly refractile crystals in tissues after autolysis had proceeded to the point where no other pathological features were recognizable. Combined with insignificant or negative results of chemical analysis of the tissues for methyl and ethyl alcohol, metallic poisons, aniline and phenolic compounds and the lack of significant gross evidence of other lesions, these findings were considered to substantiate the report that the subject had consumed "antifreeze" solution. Petri<sup>21</sup> stated that such crystals will persist in tissues for years and that they are therefore relatively stable evidence of poisoning by one of the few substances that cause their deposition.

#### GENERAL COMMENT

Observations in the cases of this report and in those of other authors indicate a number of rather specific morphological lesions which in combination are almost diagnostic of subacute poisoning with ethylene glycol. The lesions in cases of acute poisoning with this substance are sufficiently characteristic to be considered highly indicative although less specific than in the cases of subacute poisoning. The presence of large, sheaflike, brilliantly doubly refractile crystals of calcium oxalate in the renal tubules and smaller similar crystals in other tissues, particularly the leptomeninges and brain, is the most striking feature of either type of poisoning. These crystals are not specific evidence of poisoning with ethylene glycol, for Doerr<sup>8</sup> found that they occurred in animals that had been poisoned with calcium oxalate and a polyglycol ester referred to as "cremogen" ( $-\text{CH}_2\text{-COO}-(\text{CH}_2)_4\text{-COO-CH}_2-$ ), and Wiley and associates<sup>22</sup> indicated that they might be found following poisoning with ethylene glycol monoacetate, ethanol amine, glyoxal and glycollic acid. Similar crystals have been observed in the tissues of a child with an obscure metabolic disease,<sup>23</sup> but they were accompanied by extensive lesions of the bones and other organs which were quite different from the other features of the cases of poisoning with ethylene glycol. Poisoning with oxalic acid results in similar crystals,<sup>24</sup> but when that substance is ingested the corrosive effects and deposition of crystals in the gastric mucosa are distinctive features.<sup>21</sup> The crystals do not result from poisoning by propylene glycol, diethylene glycol, dioxane, various compound glycol ethers or any of the common alcohols.<sup>25</sup>

The morphological lesions of acute poisoning by ethylene glycol in addition to the crystals are nonspecific and similar to those of many other types of acute poisoning, particularly those due to alcohol. There is a small amount of fluid in the serous cavities, the lungs are markedly congested and edematous, and in other viscera there are variable degrees of congestion. The presence of microscopic evidence of diffuse interstitial inflammation, particularly in the leptomeninges, heart, stomach and diaphragm, is significant, and there is early bronchopneumonia in many cases.<sup>6</sup>

21. Petri, E.: *Pathologische Anatomie und Histologie der Vergiftungen*, in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Springer-Verlag, 1930, vol. 10, pp. 232-236.

22. Wiley, F. H.; Hueper, W. C.; Bergen, D. S., and Blood, F. R.: *J. Indust. Hyg. & Toxicol.* **20**:269, 1938.

23. Davis, J. S.; Klingberg, W. G., and Stowell, R. E.: *J. Pediat.* **36**:323, 1950.

24. Krueger, R.: *Virchows Arch. f. path. Anat.* **215**:444, 1915.

25. Geiling and Cannon.<sup>2</sup> Doerr, Kraft and Rauschke.<sup>8</sup> Wiley and others.<sup>22</sup>

In the cases of subacute poisoning in which death does not occur until late in the second week after poisoning, a peculiar focal hemorrhagic necrosis of the renal cortex and extensive hydropic changes involving the renal tubules and central zones of the liver are characteristic features. Both these lesions also occur in poisoning due to dioxane<sup>11</sup> and diethylene glycol.<sup>2</sup> The hydropic lesion of the liver has also been reported in poisoning by carbon tetrachloride,<sup>12</sup> and hydropic degeneration of the renal tubules occurs with poisoning by several of the glycol ethers.<sup>26</sup> These lesions do not occur in poisoning by calcium oxalate, and combined with the doubly refractile crystals, which do not occur following poisoning by diethylene glycol, dioxane and carbon tetrachloride,<sup>25</sup> they are relatively specific for poisoning by ethylene glycol. A widespread accumulation of fluid in interstitial tissues and serous cavities was also a feature of the two cases of subacute poisoning reported.

Interpretation of the significance of the finding of doubly refractile substance in the nuclei of cells in various tissues in several of the cases reported must await further observations.

#### SUMMARY

Two cases of subacute poisoning due to drinking "permanent-type antifreeze" (ethylene glycol) in which the subjects died 11 and 12 days after poisoning and six cases of acute poisoning due to the same substance are reported.

Focal hemorrhagic necrosis of the renal cortices and extensive hydropic degeneration of the liver and the kidneys were features of the cases of subacute poisoning not present in the cases of acute poisoning reported here and by other authors.

The crystals of calcium oxalate deposited in the renal tubules and about blood vessels in the leptomeninges are characteristic of poisoning with ethylene glycol but can result from poisoning with a few other substances. The combination of the presence of the crystals and hydropic degeneration in the liver and the kidneys is relatively specific for subacute poisoning with ethylene glycol.

A doubly refractile substance of unknown significance was observed within the nuclei of cells of various tissues in these cases.

26. Hanzlik and others.<sup>16</sup> Young, E. G., and Woolner, L. B.: *J. Indust. Hyg. & Toxicol.* 28:267, 1946.

## CONGENITAL ALVEOLAR DYSPLASIA OF THE LUNGS

NATHAN KAUFMAN, M.D.

CLEVELAND

AND

ROBERT K. SPIRO, M.D.

BROOKLYN

THE TERMS "congenital atelectasis," "fetal atelectasis" and "atelectasis of the newborn" have long served to shield ignorance about uncomplicated respiratory failure in the early neonatal period. MacMahon<sup>1</sup> described a hitherto unrecognized pathological entity which clinically is similar to fetal atelectasis. He suggested that this condition may represent a retardation or a disturbance of the normal development of the pulmonary alveoli and so named it congenital alveolar dysplasia. Inasmuch as the newly described entity may have significance in helping to explain death in the early neonatal period, a survey was made to determine the incidence and the degree of severity of congenital alveolar dysplasia in full term infants who died shortly after birth.

### METHODS

Material was gathered from the autopsy files of City Hospital during the years 1942 to 1949, Lutheran Hospital, 1946 to 1949, and Polyclinic Hospital, 1949, in Cleveland. Only the cases in which the body of the infant weighed 2,500 Gm. or more were selected. Further care was taken to eliminate all cases in which there were clinical signs and symptoms of pulmonary inflammation or in which the newborn was syphilitic or had a syphilitic mother. Thirty-seven cases remained for study.

It was necessary to depend on the protocols prepared by numerous observers for the gross descriptions of the lungs, with consequent variation in reliability. The histological study was carried out by ourselves. The number of slides available in each case varied. The tissues were fixed in solution of formaldehyde U. S. P. (formalin®) or Zenker's solution and were stained with hematoxylin and eosin and Masson's trichrome stain.

The histological picture was varied: in some instances dysplasia was prominent; in others little could be demonstrated. In order to evaluate the dysplasia, we independently reviewed the slides and recorded, according to visual microscopic impression, the degree of dysplasia. If a large portion of the lung showed dysplasia, the condition was graded "marked." If only a few areas were involved, the dysplasia was considered "slight." If the degree of involvement lay between these extremes the dysplasia was termed "moderate." In almost every instance there was complete agreement in the interpretation of the amount of dysplasia.

In order to secure a quantitation of the degree of dysplasia, the dysplastic areas were measured by a micrometric ocular. If the areas were 1.2 to 2.4 mm. in greatest dimension the dysplasia was graded "slight"; if 2.4 to 3.6 mm., "moderate," and if they were more

From the Department of Pathology, Western Reserve University School of Medicine, and Cleveland City Hospital.

1. (a) MacMahon, H. E.: Bull. New England M. Center 9:48, 1947; (b) Am. J. Path. 24:919, 1948; (c) Pediatrics 2:43, 1948.

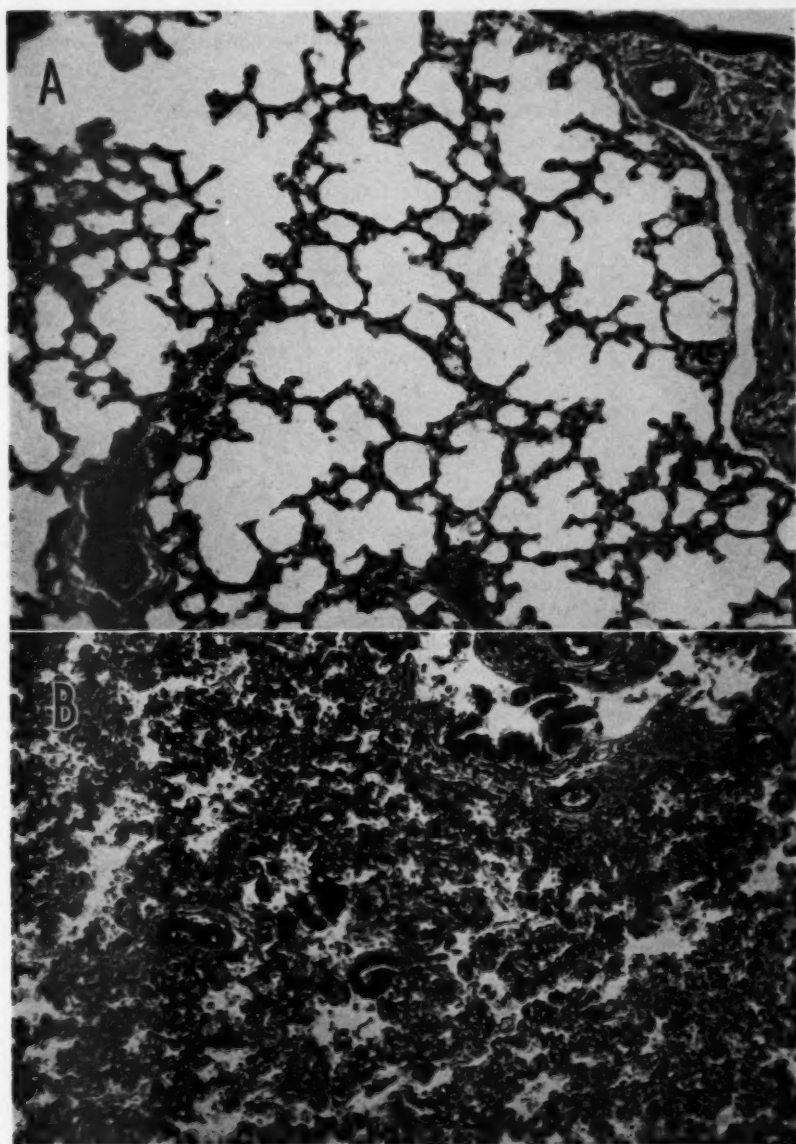


Fig. 1.—*A*, normal aerated lung of newborn;  $\times 150$ . *B*, unaerated lung of newborn, commonly referred to as fetal atelectasis;  $\times 150$ . Compare *A* and *B* with figures 2, 3 and 4.

than 3.6 mm., the dysplasia was graded "marked." In some instances numerous isolated areas between 1.2 and 2.4 mm. in greatest dimension were observed; in these the dysplasia was recorded as "slight." The evaluation of the amount of dysplasia by the visual impression technic compared very favorably with that obtained by micrometric study, although the standard for the latter technic is admittedly arbitrary. It is therefore possible to appraise effectively the amount of congenital alveolar dysplasia present by the routine method of microscopic observation.

#### ANATOMIC FINDINGS

The gross and microscopic observations of MacMahon<sup>1</sup> were confirmed. Grossly, the lungs were usually well formed, firm and purplish red. Microscopi-

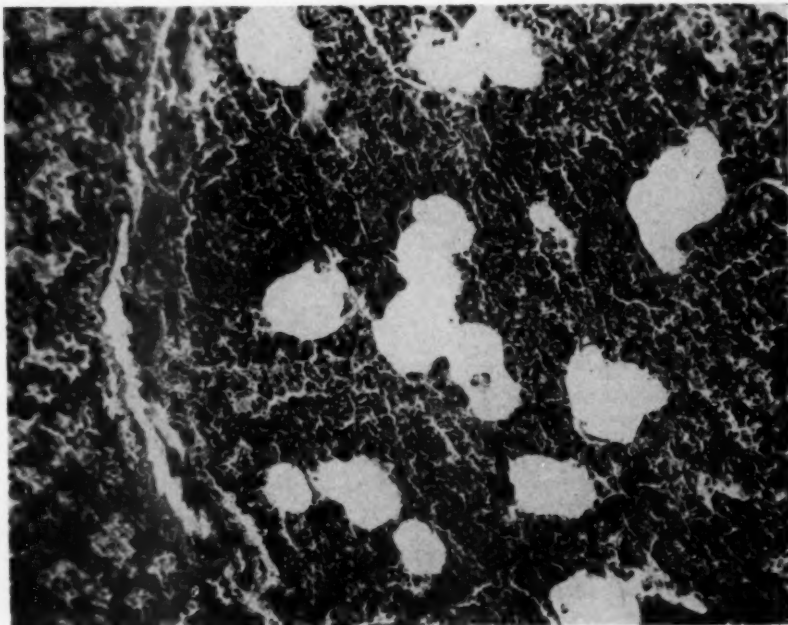


Fig. 2.—Lung showing alveolar dysplasia;  $\times 150$ . Note the small number of alveoli in the field and the thick septal walls.

cally, the areas showing the lesions did not resemble the lungs seen in the neonatal period either in the aerated (fig. 1 *A*) or in the unaerated state (fig. 1 *B*). A definite decrease was noted in the number of alveoli, and an increase in the interstitial tissue, which resembled fetal mesenchyme, being loose and cellular and not taking the stain for collagen (figs. 2 and 3). Although some of the alveolar spaces were not enlarged, others were clearly dilated (fig. 4). In spite of this dilation the alveolar walls were still many times thicker than they normally would be. The disproportion between alveoli and interstitial tissue was very striking in the well developed lesions. The alveolar septums contained a rich capillary network, and the alveolar lining resembled that seen in the mature lung rather than the

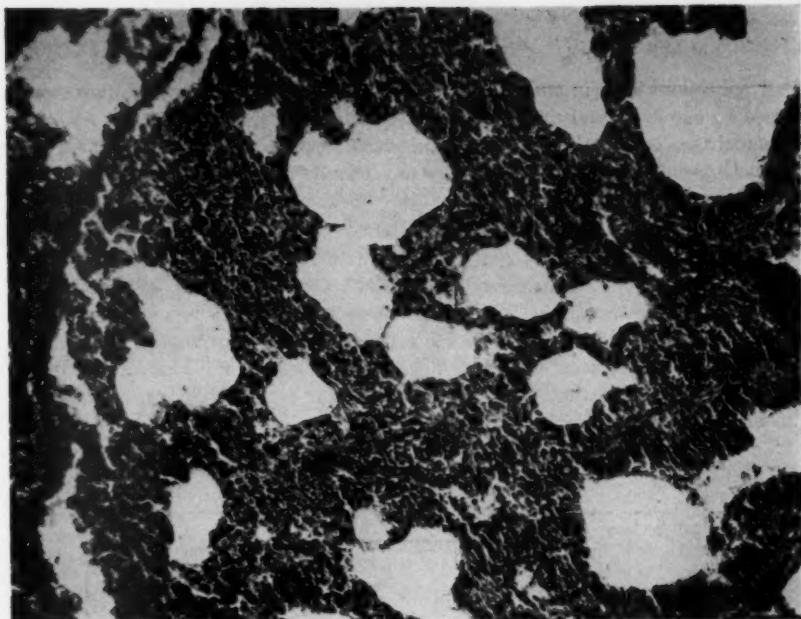


Fig. 3.—Lung showing alveolar dysplasia;  $\times 150$ . The disproportion between the alveoli and the mesenchyme is very striking.

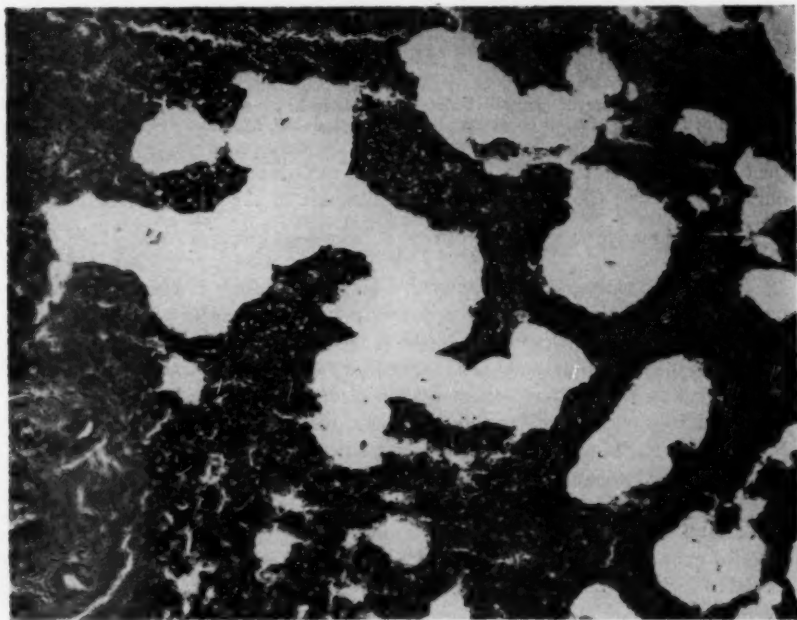


Fig. 4.—Lung showing alveolar dysplasia;  $\times 150$ . Though the alveoli are large and dilated, the alveolar septums are not thinned but are many times thicker than alveolar septums normally are.

tall epithelium seen in the immature fetus. This lesion was frequently complicated by one or several other conditions, namely, edema, congestion, intra-alveolar hemorrhage, true atelectasis, fetal atelectasis, aspiration of amniotic fluid and bronchopneumonia, all of which frequently obscured dysplastic areas, making the diagnosis rather difficult.

#### RESULTS

Congenital alveolar dysplasia was present in 23 of 37 cases studied (62 per cent). This suggests that it may be associated rather commonly with respiratory distress in the newborn (fig. 5 *A*). There were 8 girls and 15 boys with this condition. It was possible to determine the exact duration of extrauterine life in 18 of the 23 cases of dysplasia. If the one full term stillborn infant is excluded, 12 of the remaining 17 babies who breathed and had alveolar dysplasia died within 24 hours of birth (fig. 5 *B*). Of 23 with dysplasia, 7 showed a marked, 3 a moderate and 13 a slight amount (fig. 5 *C*). These figures do not represent a true incidence of

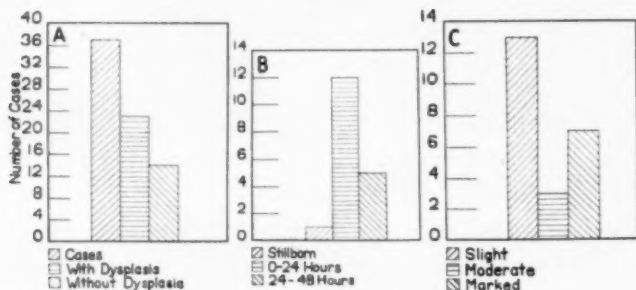


Fig. 5.—*A*, incidence of congenital alveolar dysplasia in a series of 37 selected cases; *B*, duration of life in 18 cases of congenital alveolar dysplasia; *C*, degree of dysplasia in 23 cases.

the condition, since many neonatal deaths in the records reviewed were omitted from our study because the information was inadequate or the material unsatisfactory for study.

#### COMMENT

Though the pathogenesis of congenital alveolar dysplasia is obscure, the striking feature is a marked disturbance in the normal development of the pulmonary alveoli; the other lung structures develop rather normally. Although it superficially resembles the fetal lung of the fourth or fifth month of intrauterine life and thus suggests a simple arrest or retardation of development, this resemblance does not stand up under close scrutiny. Grossly, the lung is not decreased in size. Microscopically, the immature lung presents an entirely different histologic pattern. In both, the alveoli are relatively few, there is an abundance of mesenchymal tissue, and the interlobular septums are prominent; but here the resemblance ends. The alveoli of the immature lung are arranged in a regular pattern, are lined by cuboidal epithelium and are not dilated; whereas, in congenital alveolar dysplasia the alveoli are irregularly arranged and there is irregularity in the distribution of alveoli and interstitial tissue. Some alveoli are small, while others are notably

dilated. None are lined by cuboidal epithelium; they are lined, rather, by the alveolar epithelium seen in the normal mature lung. In addition, the thickened alveolar walls are rich in capillaries, and the bronchi have the appearance of the well developed mature structure.

The recent findings of Waddell<sup>2</sup> suggest a possible explanation as to the pathogenesis of congenital alveolar dysplasia. On the basis of a study of the pulmonary development of the mammal, he presented evidence that not only the alveoli and their lining cells but the cells lining the bronchi originate from mesenchyme. He has shown that bronchial differentiation is preceded by consolidation and enlargement of mesenchymal cells. This is preceded by and associated with chemical changes in the primitive mesenchymal cells as evidenced by glycogen in the cytoplasm, followed by a change in the shape of the cell to form a cuboidal epithelium. The alveolar epithelial lining cells discharge their cytoplasmic accumulations of glycogen. In addition, some of the lining cells disintegrate in situ, others desquamate as though pushed off by the rapidly developing underlying capillaries, and others regress to form the inconspicuous cells with small nuclei and scanty cytoplasm.

If this is truly the mechanism of alveolar differentiation, then a breakdown in this mechanism could lead to a condition of alveolar dysplasia as described, so that the bronchi develop normally, as do the blood vessels, but not sufficient alveoli are formed from the mesenchyme. This would also explain the similarity of the mesenchyme in the alveolar septums in this condition and that in the immature lung. Whatever the developmental mechanism, if it is not interfered with, the final anatomic state is the one most favorable for respiratory gas exchange. At birth, most of the lung is inflated, but areas of unaerated lung can be noted for about a week. However, in disturbed development, as in congenital alveolar dysplasia, such a satisfactory state for gaseous exchange is not established, and the newborn infant is severely handicapped. The thick alveolar walls may interfere with the interchange of gases between the alveoli and the blood stream under the usual tension of the gases in the infant's lung. An additional feature in the interference with gaseous exchange is the decrease of respiratory surface due to the great decrease in the number of alveoli. MacMahon<sup>1a</sup> also feels that the lungs are less resilient as a result of this condition, and this again interferes with pulmonary aeration.

#### CONCLUSIONS

The diagnosis of congenital alveolar dysplasia may be entertained after gross examination of the lungs, but only after microscopic study can it be established. The condition is frequently accompanied with bronchopneumonia, true atelectasis, fetal atelectasis, congestion, edema, aspiration pneumonia and intra-alveolar hemorrhage, each or all of which may mask the underlying alveolar disorder.

It is of interest that this condition has not been observed in adult lungs. The only explanation offered is that these areas of dysplasia, if the infant survives, are prone to repeated infection and hence fibrosis, so that the basic lesion is obscured or even obliterated.

2. Waddell, W. R.: Organoid Differentiation of the Fetal Lung: A Histologic Study of the Differentiation of Mammalian Fetal Lung in Utero and in Transplants, *Arch. Path.* **47**:227 (March) 1949.

Congenital alveolar dysplasia appears to be a heavy handicap for the newborn. Of 17 who had the condition and who breathed, 12 died within 24 hours of birth (fig. 5 B). The disadvantage to respiration is perhaps greatest in the first few hours of life while the infant converts its circulation-respiration balance from one dependent on the mother to an independent one.

#### SUMMARY

Congenital alveolar dysplasia has been found to some degree in 23 (62 per cent) of 37 full term infants who died within 48 hours of birth and were submitted to postmortem examination.

The degree of dysplasia was marked in 7, moderate in 3 and slight in 13.

Of the babies who breathed and had alveolar dysplasia, 71 per cent died within 24 hours of birth.

## CHANGES IN THE SERUM LIPIDS OF RABBITS WITH ACUTE URANYL NITRATE POISONING

FREDERICK C. BAUER Jr., M.D.

GRANT C. JOHNSON, M.D.

LYNN CARBONARO, B.S.

AND

EDWIN F. HIRSCH, M.D.

CHICAGO

CHANGES occur in the lipid content of the blood in patients with nephritis, in animals with nephritis experimentally produced with mercury bichloride, uranyl nitrate, potassium dichromate and nephrotoxic antikidney serum, and in animals subsequent to unilateral and bilateral nephrectomy. Heymann and Clark<sup>1</sup> observed an increase of the serum lipids in dogs and rats poisoned with mercury bichloride but found none in rabbits so poisoned.

Changes in tissues with uranium poisoning have been studied in many animals with particular attention to renal damage,<sup>2</sup> but only a few studies have been made of the blood lipids in these animals. Bing, Heckscher and Jessen<sup>3</sup> in 1925 injected uranyl acetate into rabbits and observed a moderate but inconstant elevation of blood cholesterol. Politzer<sup>4</sup> poisoned rabbits with uranyl acetate and found an increase in the blood neutral fat, cholesterol and phospholipid. Heymann and Clark<sup>1</sup> reported an increase in total lipids and cholesterol in dogs poisoned with uranium. The comprehensive report edited by Voegtlin and Hodge<sup>2c</sup> stated that in rats poisoned with uranium the cholesterol of the blood, after a decrease on the first day, rose to 1.12 times the control level and remained that high for about two weeks. In humans accidentally poisoned by uranium no significant change was observed

Dr. Johnson is John Jay Borland Fellow, St. Luke's Hospital.

Miss Carbonaro is Seymour Coman Fellow in the Department of Pathology, University of Chicago.

This investigation was aided by the Winfield Peck Memorial Fund.

From the Henry Baird Favill Laboratory, St. Luke's Hospital, and the Department of Pathology, University of Chicago.

1. Heymann, W., and Clark, E. C.: *Am. J. Dis. Child.* **70**:74, 1945.

2. (a) Simonds, J. P., and Hepler, O. E.: *Experimental Nephropathies: Method of Producing Controlled Selective Injury of Renal Units by Means of Chemical Agents*, *Arch. Path.* **39**:103 (Feb.) 1945. (b) Hunter, W. C., and Roberts, J. M.: *Am. J. Path.* **8**:665, 1932. (c) Horn, H.: *Experimental Nephropathies*, *Arch. Path.* **23**:71 (Jan.) 1937. (d) Hunter, W. C.: *Ann. Int. Med.* **1**:747, 1928. (e) Voegtlin, C., and Hodge, H. C.: *Pharmacology and Toxicology of Uranium Compounds*, New York, McGraw-Hill Book Company, Inc., 1949, pp. 221, 258, 268 and 1003.

3. Bing, H. I.; Heckscher, H., and Jessen, J.: *Acta path. et microbiol. Scandinav.* **2**:234, 1925.

4. Politzer, M.: *Arch. di farmacol. sper.* **62**:70, 1936.

in the cholesterol of the blood, although the blood nonprotein nitrogen increased. The time when the determinations of the cholesterol of the blood were made and the number of determinations are not mentioned by Voegtlin and Hodge.

#### MATERIAL AND METHODS

The published reports on the changes of the serum lipids in rabbits poisoned with uranium are incomplete or in disagreement. With the hope that these conflicting results could be clarified, a reinvestigation of the lipid content of the serum of rabbits with uranium poisoning was made. Serum lipids and nonprotein nitrogen were determined daily for one to three days preceding and at one to three day intervals following the injection of uranyl nitrate (uranium nitrate, Merck,  $UO_2(NO_3)_2 \cdot 6H_2O$ ). Total serum esterified fatty acids were determined by the method of Bauer and Hirsch.<sup>5</sup> Cholesterol and cholesterol ester were determined by the method of Schoenheimer and Sperry<sup>6</sup> except when total cholesterol was determined by Sackett's<sup>7</sup> modification of Bloor's method adapted for spectrophotometric analysis. After the lipid extract had been digested with sulfuric acid and hydrogen peroxide, the lipid phosphorus was determined by the method of Fiske and Subbarow<sup>8</sup> adapted to spectrophotometric analysis. Milliequivalents of fatty acid combined with lipid phosphorus were calculated according to the formula described by Peters and Man<sup>9</sup>:

Phospholipid fatty acid (mEq/L.) = lipid phosphorus (mEq./l.)  $\times$  1.8. Neutral fat was calculated as follows:

Neutral fat = total esterified fatty acid - (cholesterol ester fatty acid + phospholipid fatty acid). All values were expressed as milliequivalents per liter (mEq./l.).

Any effect produced on the blood lipids by the loss of the blood removed for chemical analysis was controlled by bleeding another rabbit simultaneously and in the same amounts as those removed from the uranium-poisoned animals. No significant variations of the nonprotein nitrogen, the serum lipid or the protein content resulted from these bleedings.

The rabbits were fed a balanced ration for at least one week preceding the experiment and during it.

#### RESULTS

In rabbits receiving a subcutaneous injection of 10 mg. of uranyl nitrate there was transient elevation of the serum esterified fatty acid, cholesterol, cholesterol ester, lipid phosphorus and nonprotein nitrogen for two or three weeks. Plotted in chart 1 are the variations in the serum cholesterol and fatty acid levels of five animals following such an injection. The total serum esterified fatty acid increased appreciably. The increase of the total cholesterol was smaller. In chart 2 the levels of the various lipid fractions are presented graphically as determined in rabbit 7 after the subcutaneous injection of 10 mg. of the uranyl nitrate. Although all fractions increased at about the same time, the maximum increase was in the esterified fatty acid fraction, attributed to neutral fat. The nonprotein nitrogen of the blood increased at about the same time as the lipids but returned to or near the control level a few days before the lipids. This also occurred in other rabbits (5, 9 and 11).

In rabbits 1, 2, 3 and 5 a slight decrease in serum esterified fatty acids occurred between five and 24 hours after the uranium injection. In rabbits 1, 3, 4 and 5 there was a similar drop in cholesterol. In most of the other animals blood was not

5. Bauer, F. C., Jr., and Hirsch, E. F.: Arch. Biochem. **20**:242, 1949.

6. Schoenheimer, R., and Sperry, W. M.: J. Biol. Chem. **106**:745, 1934.

7. Sackett, G. E.: J. Biol. Chem. **64**:203, 1925.

8. Fiske, C. H., and Subbarow, Y.: J. Biol. Chem. **66**:375, 1925.

9. Peters, J. P., and Man, E. B.: J. Clin. Investigation **22**:707, 1943.

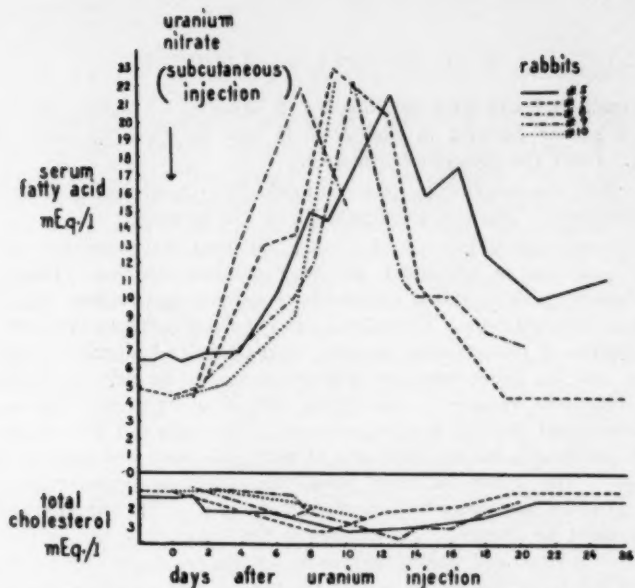


Chart 1.—Changes in total serum esterified fatty acid and total cholesterol in five rabbits following subcutaneous injection of uranyl nitrate (Merck's uranium nitrate), 5 mg. in rabbit 5 and 10 mg. in rabbits 7, 8, 9 and 10.

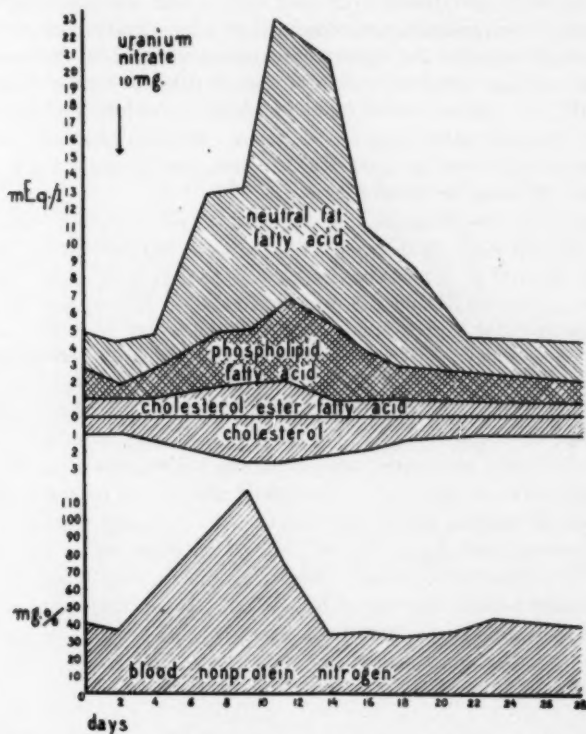


Chart 2.—Changes in serum lipid fractions and blood nonprotein nitrogen in rabbit 7 following subcutaneous injection of 10 mg. of uranyl nitrate (Merck's uranium nitrate).

analyzed until 24 hours after the injection of uranium. Voegtlin and Hodge<sup>26</sup> reported a similar decrease in cholesterol in rats the first day after uranium poisoning. Later the cholesterol increased.

The rabbits' consumption of food was markedly reduced during the period of renal insufficiency. This was accompanied by a loss in weight.

One animal was killed one day after attaining the maximum elevation of serum lipids and on the eighth day after uranium injection. Tissues were fixed in Zenker's solution and in formaldehyde solution, and sections were stained with hematoxylin and eosin. The cells of the convoluted tubules of the kidney had varying degrees of retrogressive changes. Some tubules had only the basement membrane, and the lumen contained amorphous acidophilic debris. Occasionally isolated lining cells remained. The lining cells of other tubules seemed to be intact. Glomerular changes were not present. The cords and the lobules of the liver were unchanged, but the cytoplasm of many cells contained an abundance of fat vacuoles. The spleen had large phagocytes containing brown granular iron pigment (prussian blue microchemical reaction for iron). The pancreas and other tissues revealed no changes. In sections of the kidney and the liver fixed in formaldehyde solution and stained with sudan IV the cytoplasm of the cells of the convoluted tubules of the kidney and of the hepatic cells contained quantities of lipid droplets.

#### COMMENT

Bing, Heckscher and Jessen<sup>3</sup> poisoned rabbits with multiple small doses of uranyl acetate. The maximum total dose was 40.6 mg. divided into 71 injections over a period of 99 days. An inconstant elevation occurred in blood cholesterol. The maximum change was from a control level of 0.04 per cent to 0.34 per cent (Gm. per 100 cc.). Serum neutral fat and phospholipid values were not recorded.

Politzer<sup>4</sup> poisoned rabbits with uranyl acetate. Between four and 11 days later he found the blood neutral fat increased 3.3 times, free cholesterol 2.3 times and phospholipid 1.77 times the control levels.

After injecting uranyl nitrate into dogs, Heymann and Clark<sup>1</sup> observed an increase of the total serum lipids from a level between 500 and 800 mg. per 100 cc. to 1,020 mg. per 100 cc. just before death about 10 days later. The total cholesterol rose from between 84 and 125 mg. per 100 cc. to 225 mg. per 100 cc., and the free cholesterol rose slightly from between 29 and 58 mg. per 100 cc. to 59 mg. per 100 cc. Accordingly, the increase of cholesterol was in the esterified portion. The amounts of serum phospholipid and neutral fat were not recorded for these animals. No dog survived the uranium poisoning, so that information is not available concerning the serum lipid levels beyond 10 days after the injection of uranium. The serum lipids and nonprotein nitrogen of our rabbits surviving the uranium poisoning approached normal levels and remained near normal for many weeks.

The report of Voegtlin and Hodge<sup>26</sup> on uranium poisoning regards the kidney as the only parenchymal organ affected. Effects on other organs are considered consequent to acidosis and azotemia. Regarding rabbits, they reported that liver fat was increased without necrosis of hepatic cells, but in dogs some necrosis of hepatic cells accompanied the increase in liver fat.

The simultaneous, although transient, elevation of the serum lipid fractions and nonprotein nitrogen, the fatty changes of the liver and the kidney, and the retrogressive changes of the kidney suggest that a systemic disturbance of lipid metabolism resulted from the uranium poisoning. Because the rabbits consumed less than the usual amount of food during the lipemic phase, the increased amount of serum lipids is not due directly to food intake but comes rather from lipid depots within the body. Therefore, some factor controlling mobilization of stored lipid is disturbed by the uranium poisoning. The triglycerides of depot fat<sup>10</sup> can be the source of the increased amounts of neutral fat which appear in the serum.

Popjak<sup>11</sup> determined quantitatively the lipids of human kidneys and found with fatty changes an increase in the amount of neutral fat. Phospholipids and cholesterol were not increased. He concluded that fatty changes of the kidney represent an infiltration of renal tissues by neutral fat derived from the fat depots. Dible and Popjak<sup>12</sup> produced fatty changes in kidneys of rabbits by starvation. On the basis of reduction in iodine value, increase in quantity and relationship of the increase of kidney lipid to the amount of depot fat they also concluded that the fatty changes of the kidney represent an infiltration of lipids from the fat depots.

Dible and Libman<sup>13</sup> produced fatty livers in rabbits by starvation and concluded that the extent of fat infiltration of the liver is determined by the amount of available depot fat, thus implying that the lipids are derived from that source. In patients with nephrotic lipemia Ahrens<sup>14</sup> found a large increase of the serum neutral fat.

The changes produced in rabbits by uranium poisoning in some respects are similar to the so-called lipid nephrosis of humans. In both, there are fatty changes of the renal tubules with some changes of the glomeruli<sup>2b</sup> and hyperlipemia.<sup>15</sup> In both, the serum neutral fat is greatly increased.

#### SUMMARY

Uranyl nitrate poisoning in rabbits was associated with transient azotemia and lipemia of two to three weeks' duration. All lipids participated in this increase, but the greatest increase was in the neutral fat fraction. The nonprotein nitrogen returned to near the control level several days before the lipids. The significance of these results with other forms of nephritis is discussed.

10. Peters, J. P., and Van Slyke, D. D.: *Quantitative Clinical Chemistry Interpretations*, Baltimore, Williams & Wilkins Company, 1946, vol. 1, p. 376.

11. Popjak, G.: *J. Path. & Bact.* **57**:87, 1945.

12. Dible, J. H., and Popjak, G.: *J. Path. & Bact.* **53**:133, 1941.

13. Dible, J. H., and Libman, J.: *J. Path. & Bact.* **38**:269, 1934.

14. Ahrens, E. H., Jr.: *Bull. New York Acad. Med.* **26**:151, 1950.

15. Bell, E. T.: *Renal Diseases*, Philadelphia, Lea & Febiger, 1947, p. 198.

## THORACIC VENOUS ANOMALIES

### III. Atresia of the Common Pulmonary Vein, the Pulmonary Veins Draining Wholly Into the Superior Vena Cava (Case 3)

### IV. Stenosis of the Common Pulmonary Vein (Cor Triatriatum) (Case 4)

JESSE E. EDWARDS, M.D.

JAMES W. DuSHANE, M.D.

DONALD L. ALCOTT, M.D.

AND

HOWARD B. BURCHELL, M.D.

ROCHESTER, MINN.

**I**N AN EARLIER communication two of us (Edwards and DuShane<sup>1</sup>) reported on two cases of unusual anomalies of the thoracic veins related to abnormal development of the pulmonary venous system. Since that report was made, we have observed two additional examples of thoracic venous anomalies. While the gross anatomic and the functional abnormalities are quite dissimilar in the two newer cases, these two cases are closely related to each other developmentally. In one there was an atretic common pulmonary vein associated with a pulmonary venous system that drained completely into the superior vena cava. In the other there was an apparent third atrial chamber (cor triatriatum) caused, in our opinion, by stenosis of the common pulmonary vein. These two cases logically fall into a series with the two cases reported in the earlier communication. For this reason and to avoid confusion the cases of this communication will be referred to as cases 3 and 4, respectively, of the series of four cases reported in the two papers. To maintain continuity of this report with the first, the illustrations will run in sequence with those of the first report. Consequently, the first figure of this communication will be designated as figure 21.

#### CASE 3

*Clinical Features.*—A boy 5½ weeks of age was admitted to the hospital on July 4, 1950 because of rapid, noisy breathing and tilting of the head to the left. Birth had been medically induced because the mother had uterine fibroids. After delivery this baby had been blue and breathed rapidly. Oxygen was administered for 24 hours. The rapid breathing gradually subsided but recurred when the baby cried or took his feedings.

Dr. Alcott is Fellow in Pathology, Mayo Foundation.

From the Section on Pathologic Anatomy (Dr. Edwards), the Section on Pediatrics (Dr. DuShane) and the Division of Medicine (Dr. Burchell), of the Mayo Clinic.

1. Edwards, J. E., and DuShane, J. W.: Thoracic Venous Anomalies: I. Vascular Connection of the Left Atrium and the Left Innominate Vein (Levoatriocardinal Vein) Associated with Mitral Atresia and Premature Closure of the Foramen Ovale (Case 1); II. Pulmonary Veins Draining Wholly into the Ductus Venosus (Case 2), Arch. Path. 49:517 (May) 1950.

Physical examination disclosed a flabby infant whose head was tilted to the left. Respirations were rapid. Cyanosis was observed when he cried. Some edema was noted about the neck and lower jaw. Roentgenograms of the thorax showed bilateral emphysema (fig. 21). The electrocardiogram demonstrated right ventricular hypertrophy (fig. 22). Esophagrams revealed no evidence of obstruction or deformity. The results of urinalysis were normal. Erythrocytes numbered 3,670,000 per cubic millimeter of blood and there was 12.2 Gm. of hemoglobin per 100 cc. of blood.

After the baby was admitted to the hospital, cyanosis became more pronounced in spite of constant administration of oxygen. The edema of the neck and lower jaw increased, extending to the face and scalp. Respirations were noisy, with an expiratory grunt, and feedings were taken with considerable difficulty. A venous anomaly was suspected, and venography was performed, 5 cc. of iodopyracet injection U. S. P. (solution diodrast® 35 per cent W/V) being injected into the longitudinal sinus. The roentgenograms showed the left transverse sinus and

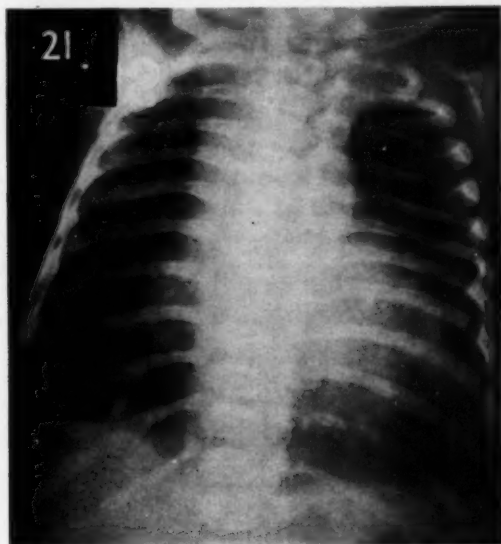


Fig. 21 (case 3).—The roentgenogram of the thorax. Bilateral pulmonary emphysema.

the left jugular vein to be smaller than the right. Soon after the venogram was made, the infant died, on July 17, 1950, at the age of 7½ weeks.

*Pathologic Features.*—Emerging from the left lung were two veins which converged to form one left pulmonary vein, and emerging from the right lung were three veins which converged to form a single right pulmonary vein. The single right and left pulmonary veins each became continuous with a sinus-like sac measuring 1.1 cm. in height and 1.5 cm. in lateral dimensions (figs. 23 through 25). The sac lay superior to the left atrium, inferior to the bifurcation of the trachea, anterior to the esophagus and posterior to the right and left pulmonary arteries. From the right upper angle of the sinus a venous channel, measuring 3 mm. in length and 5 mm. in diameter, proceeded forward to join the superior vena cava just above its entrance into the right atrium and 1.3 cm. inferior to the entrance of the azygos vein into the superior vena cava (fig. 25). From the lowermost portion of the venous sinus at about the midline of the body, a cordlike strand, measuring 0.6 cm. in length and 1.5 mm. in diameter, extended to the dorsal wall of the left atrium. The lowermost part of the strand contained a lumen, but the upper half was atretic (figs. 23 through 25).

The entire circumference of that portion of the venous sinus into which the atrietic strand inserted was thick walled and brownish gray. This zone measured 1.0 cm. from left to right. It resembled atrial wall with regard to texture and color. In a small zone measuring 0.5 cm. in diameter the right inferior portion of the venous sinus had a similar appearance. Microscopic examination of one of these foci revealed a layer of cardiac muscle separated from the

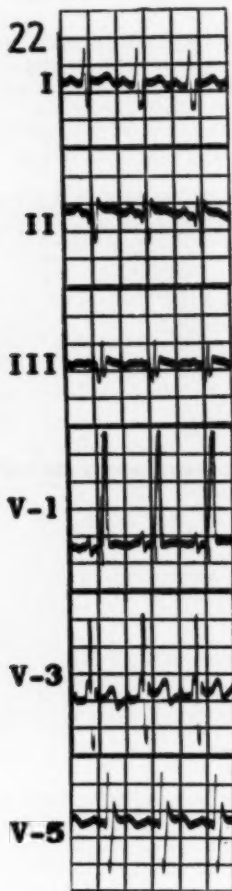


Fig. 22 (case 3).—The electrocardiogram. Right ventricular hypertrophy.

lumen by a layer of collagenous and elastic tissue resembling endocardium (figs. 26 and 27). Elsewhere the pulmonary veins and the pulmonary venous sinus were opaque gray and had the microscopic appearance of pulmonary vein.

The heart showed evident enlargement of the right-sided chambers. The right atrial wall was hypertrophied, measuring as much as 2 mm. in thickness. The right ventricular wall was hypertrophied, measuring from 0.5 to 0.7 cm. in thickness. The left atrium was small as compared with the right atrium and smaller than the anticipated normal. Its wall was thin, measuring

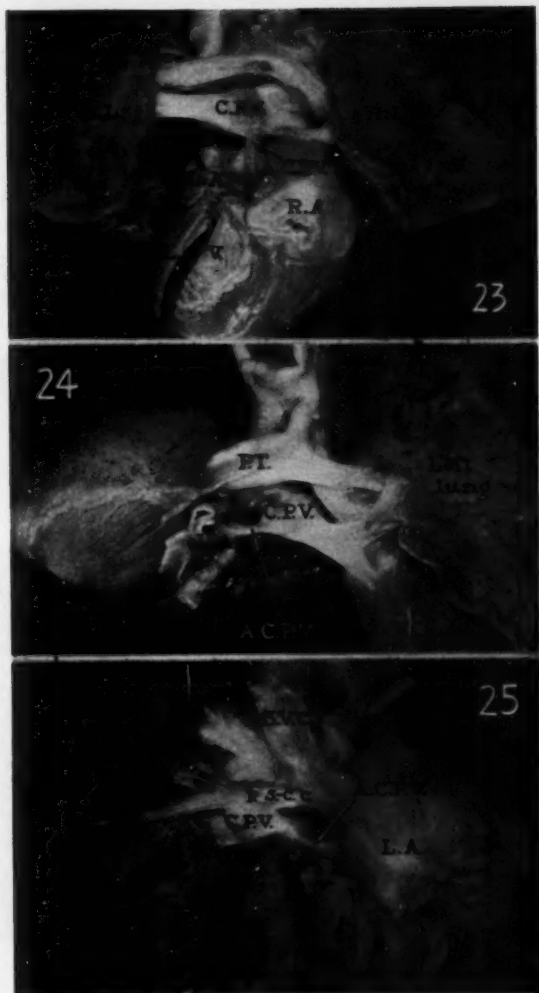


Fig. 23 (case 3).—Posterior view of the thoracic organs. The pulmonary veins converge into a common pulmonary vein (*C. P. V.*). From the lower aspect of this structure a cord (*A. C. P. V.*) considered to be an atretic segment of the common pulmonary vein, runs to the left atrium (*L. A.*). *L. L.* is the left lung; *R. L.*, the right lung; *R. A.*, the right atrium; *L. V.*, the left ventricle. The dark discoloration of the common pulmonary vein immediately above the atretic cord represents a grossly thickened zone. Microscopic sections from this region are shown in figures 26 and 27.

Fig. 24 (case 3).—Left anterior view of the thoracic organs. The heart has been reflected to the right. The veins of the left lung are seen entering the common pulmonary vein (*C. P. V.*). From the lower aspect of the common pulmonary vein a cord (*A. C. P. V.*) extends to the left atrium (*L. A.*). *P. T.* is the pulmonary trunk.

Fig. 25 (case 3).—Right anterior view of the thoracic organs. The heart has been reflected to the left. The right pulmonary veins enter the common pulmonary vein (*C. P. V.*). The atretic strand (*A. C. P. V.*) extending from the lower aspect of the common pulmonary vein to the left atrium (*L. A.*) is shown. From the upper right aspect of the common pulmonary vein a vein emerges (*P. S.-C. C.*) and enters the superior vena cava (*S. V. C.*). This communication between the common pulmonary vein and the superior vena cava is considered to be a persistent communication between the splanchnic plexus and the cardinal venous system. See figures 28 and 29.

not more than 1 mm. in thickness. The left ventricular chamber appeared to be somewhat smaller than normal. The wall measured from 0.4 to 0.6 cm. in thickness. The foramen ovale was widely patent, although guarded by an adequate valve. The ventricular septum was intact. The great vessels were properly interrelated. The ductus arteriosus had a firm, cordlike character consistent with the appearance of a closing ductus. There were no malformations of the aortic arch. The coronary arteries arose from the aorta.

The left transverse cranial venous sinus and the left internal jugular vein were narrow, each measuring about 2 mm. in diameter. The corresponding veins on the right were normal. The brain was normal.

The lungs were mottled, alternating reddish purple with pinkish gray. Microscopically, the lungs showed diffuse congestion and focal alveolar hemorrhages. In many alveolar spaces

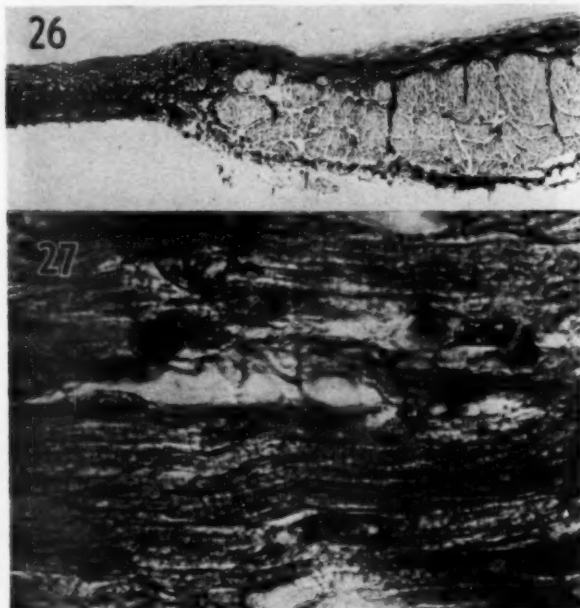


Fig. 26 (case 3).—Thickened region in the common pulmonary vein showing essentially the structure of cardiac wall merging with the structure of pulmonary vein. (Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain;  $\times 30$ .)

Fig. 27 (case 3).—Thickened portion of the common pulmonary vein showing cardiac muscle. (Mallory's phosphotungstic acid-hematoxylin stain;  $\times 1,040$ .)

there were numerous macrophages, some of which were vacuolated and some of which contained golden brown granules. The intrapulmonary arteries were normal for a person of the patient's age.

The liver and the kidneys were normal microscopically.

#### COMMENT ON CASE 3

The pulmonary and the intracardiac circulation and the developmental basis of the malformations in case 3 are portrayed diagrammatically in figures 28 and 29, respectively.

Auër<sup>2</sup> demonstrated in the human embryo that from the portion of the sinoatrial region which is destined to become the left atrium an evagination occurs which advances into the dorsal mesocardium to become the common pulmonary vein. The evagination is directed toward the pulmonary primordia. In the latter region and in the nearby esophagus there is a plexus of capillaries, the splanchnic plexus.<sup>3</sup> The splanchnic plexus connects, on the one hand, with the systemic or cardinal veins

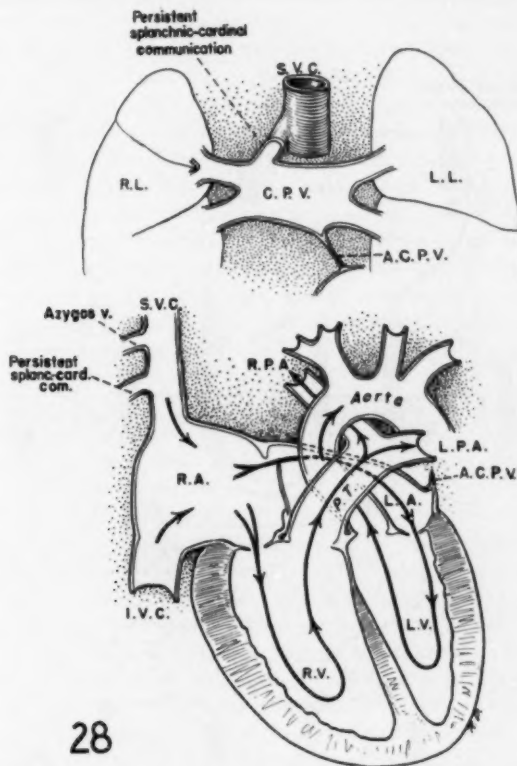


Fig. 28 (case 3).—The upper part of the figure shows the connections of the individual pulmonary veins with the common pulmonary vein (C. P. V.). The latter in turn communicates by means of a venous channel, considered to be a persistent splanchnic venous-cardinal venous communication, with the superior vena cava (S. V. C.). In the lower part of the illustration the intracardiac circulation is shown. No blood enters the left atrium through venous communications. All of the blood entering the heart is received by the right atrium. Part of this blood flows through the tricuspid valve, while part enters the left atrium through a patent foramen ovale. A. C. P. V. is the atretic lower portion of the common pulmonary vein.

and, on the other, with the common pulmonary vein. Eventually, from this basic arrangement, a vein drains each of the pulmonary lobes into the common stem

2. Auër, J.: *Anat. Rec.* **101**:581, 1948.

3. Brown, A. J.: *Anat. Rec.* **7**:299, 1913.

connecting with the atrial portion of the heart. By a process of differential growth, the common pulmonary vein becomes incorporated into the dorsal wall of the left atrium, and this results in the definitive condition wherein usually four pulmonary veins enter the left atrium independently.

As the common pulmonary vein is incorporated progressively into the wall of the left atrium, most of the connections of those parts of the splanchnic plexus which become differentiated into pulmonary vessels lose their connections with the cardinal veins.

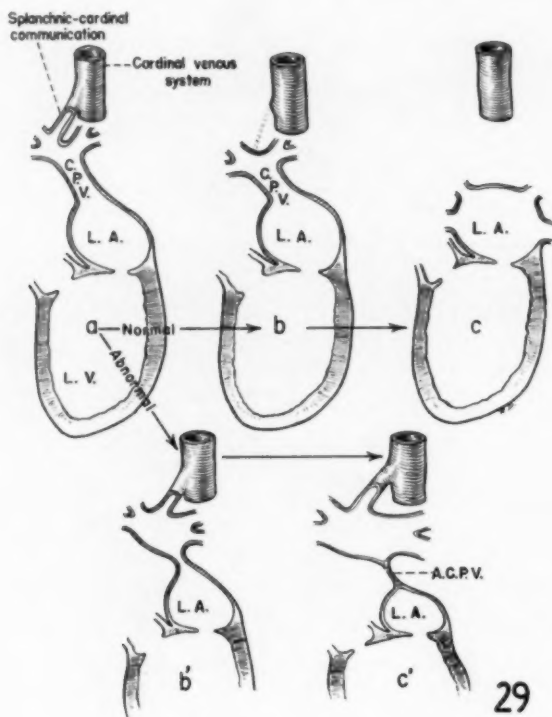


Fig. 29 (case 3).—The developmental basis of the malformation in case 3. In the upper row are illustrations showing normal evolution of the pulmonary veins. At an early stage (a) there are connections between the splanchnic plexus representing the primordia of the pulmonary veins, on the one hand, and the cardinal system of veins, on the other. The pulmonary veins join a common trunk, the common pulmonary vein (C.P.V.), which is an evagination of the sinoatrial region of the heart. Eventually the communications between the splanchnic plexus and the cardinal veins are lost (b), while, by differential growth, the common pulmonary vein becomes incorporated into the wall of the left atrium. This results in the definitive condition wherein the pulmonary veins enter the left atrium independently (c). In the lower part of the illustration is represented the interpretation that the basis of the malformation was secondary atresia of the lower aspect of the common pulmonary vein (b', c'). The upper aspect remained patent to receive the pulmonary veins. The connection between the patent portion of the common pulmonary vein and the superior vena cava represents persistence of a communication between the splanchnic plexus and the cardinal system of veins. A.C.P.V. is the atretic lower portion of the common pulmonary vein; L.A., the left atrium; L.V., the left ventricle.

In our case 3 the malformation seems to depend on faulty development of the common pulmonary vein. Either the evagination of the sinoatrial region of the heart that was to become this vein failed to establish communications with the splanchnic plexus or, if these connections were established, the common pulmonary vein became secondarily atretic. The latter explanation, that part of the common pulmonary vein became atretic after connections between the common pulmonary vein and the splanchnic plexus of vessels had become established, seems the appropriate one. In keeping with this assumption is the cardiac muscle which was present in the wall of the sinus into which the pulmonary veins drained. The sinus is interpreted as representing a dilated cranial end of the common pulmonary vein, while the strand of tissue which ran from the caudal end of the sinus to the dorsal wall of the left atrium is considered as the atretic part of the common pulmonary vein. Atresia of the lower portion of the common pulmonary vein must have occurred at a relatively early age, at a time when at least some of the connections between the splanchnic plexus and the cardinal venous system were still present. The connection between the common pulmonary venous sinus and the superior vena cava is interpreted as representing a retention and elaboration of a splanchnic-cardinal communication. The persistence of this communication seems to depend on the fact that as the common pulmonary vein became atretic the communication carried all of the blood returning from the lungs and so failed to become obliterated as it normally does.

Mention should be made of the analogous nature of the vein connecting the common pulmonary vein and the superior vena cava in case 3 and the levoatriocardinal vein in case 1 of this series. In the latter case there were mitral atresia and premature closure of the foramen ovale. The only outlet for pulmonary venous blood was a vein extending from the left atrium to the left innominate vein. This anomalous connection was named a levoatriocardinal vein. The developmental basis for it was explained as follows: An early connection between a pulmonary vein and a portion of the cardinal system of veins had been retained. As the pulmonary veins became incorporated into the left atrium in a normal manner, this retained connection was likewise carried into the wall of the left atrium. In our case 3 the atresia of a portion of the common pulmonary vein precluded the incorporation of the functioning portion of the common pulmonary vein, but, as in case 1, an early connection between a pulmonary vein and a portion of the cardinal system had been retained secondary to a barrier to the normal flow of pulmonary venous blood.

Aside from the atretic strand of common pulmonary vein in our case 3, that case is like other reported examples in which the entire pulmonary venous system drains into the right atrium or one of its tributary veins. In these cases all of the incoming blood, venous as well as oxygenated, enters the right atrium (fig. 28). From this chamber part of the mixture flows through the tricuspid valve to the right ventricle and then to the lungs. A portion of the blood in the right atrium flows through the patent foramen ovale into the left atrium and then through the left ventricle to the aorta for distribution to the systemic circulation. It is obvious that the blood delivered to the systemic circulation, as well as that to the lesser circulation, is a mixture of venous and oxygenated blood.

In our case 3, as in all cases in which there is complete anomalous drainage of the pulmonary veins into the right atrium or one of its tributaries, there are both arteriovenous and venous-arterial shunts. With regard to the types of shunts, such cardiovascular systems function as though the heart were but two-chambered. It has been emphasized that in this regard malformations such as these function differently from those in which only part of the pulmonary veins drain anomalously into the right atrium or one of its tributaries while the rest of the veins enter the left atrium normally. In partial anomalous pulmonary venous drainage there is only an arteriovenous shunt, the functional disturbance of the malformation being similar to that of atrial septal defect.

The early death in our case 3, wherein all of the pulmonary venous blood drained anomalously, is consistent with the early death in our case 2, in which the pulmonary veins drained wholly into the ductus venosus, and with the cases reported and reviewed in the classic paper by Brody<sup>4</sup> and in the contributions of others.

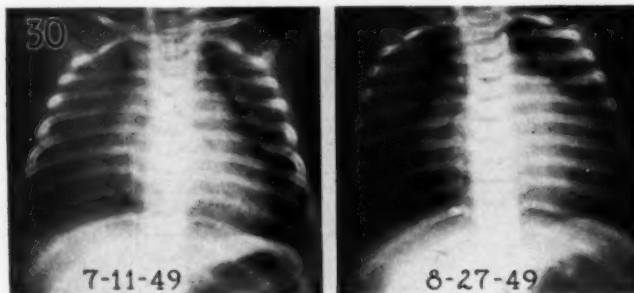


Fig. 30 (case 4).—Roentgenogram of the thorax. (a) At 5 months of age there was questionable cardiac enlargement. (b) At 6½ months of age the heart definitely had enlarged.

#### CASE 4

*Clinical Features.*—An infant girl, born at full term after an uneventful pregnancy, was admitted to the hospital on Nov. 1, 1949, at the age of 6½ months. She had vomited frequently and cried almost every night since birth, but had progressed satisfactorily until five days before she was admitted to the hospital. At this time she had acute otitis media and received penicillin. During this illness there were several episodes of severe crying and dyspnea associated with pallor and generalized rigidity.

Physical examination disclosed an infant acutely ill, without definite cyanosis. The skin was pale. The extremities were flaccid. No deep reflexes were obtained, although some spontaneous movement of the limbs was observed. The anterior fontanel was soft. The cardiac rate was rapid, being 144 beats per minute. No murmurs were noted. The liver was enlarged, extending almost to the right iliac crest.

The infant's color improved with administration of oxygen, and she took water and feedings of milk satisfactorily the night of admission. She was found dead in her crib early the following morning before laboratory studies were undertaken. Roentgenograms of the thorax had revealed progressive enlargement of the heart (fig. 30). Dr. W. Norman Doss, of Leon, Iowa, submitted the roentgenograms.

4. Brody, H.: Drainage of Pulmonary Veins into Right Side of Heart, *Arch. Path.* **33**:221 (Feb.) 1942.

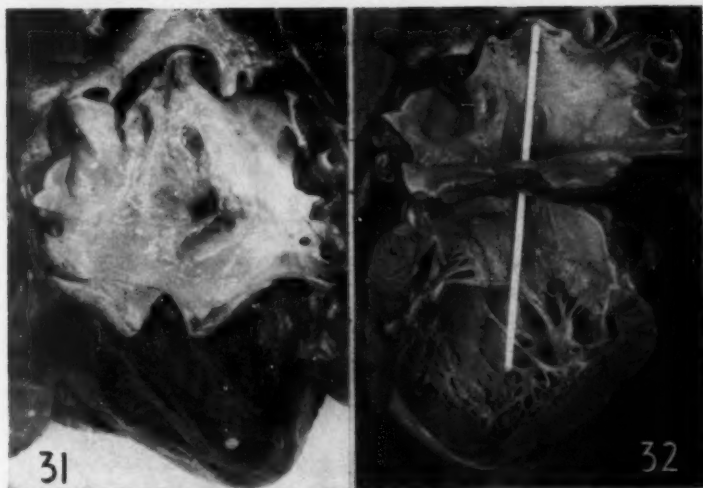


Fig. 31 (case 4).—Superior view of the opened chamber which receives all of the pulmonary veins. At the lower aspect and center of this chamber there is a small opening through which the chamber communicates with the left atrium. See figure 32.

Fig. 32 (case 4).—The left side of the heart and the accessory chamber which receives the pulmonary veins. The probe is in the small opening which connects the accessory chamber with the true left atrial chamber. The mitral valve is normal.

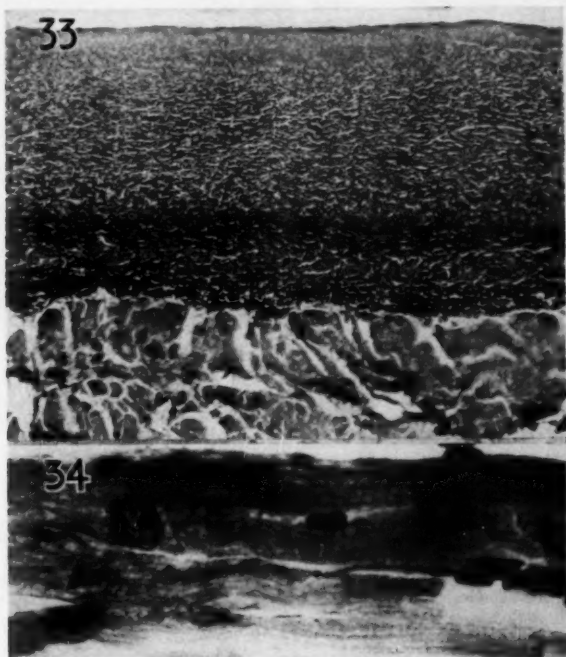


Fig. 33 (case 4).—Section through the wall of the accessory chamber, showing the structure of cardiac wall. The endocardium is thickened by collagen and elastic tissue. (Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain;  $\times 60$ .)

Fig. 34 (case 4).—Wall of the accessory chamber, showing cardiac muscle. (Mallory's phosphotungstic acid-hematoxylin stain;  $\times 1,040$ .)

*Pathologic Features.*—All of the pulmonary veins drained into a common chamber measuring 1.3 cm. in its superoinferior dimension (fig. 31). The lining of the common chamber was opaque and gray, resembling thickened endocardium. The wall of this chamber was composed of this thickened lining and an outer muscular layer, the two together measuring 3 mm. in thickness. In the inferior wall of this chamber there was an opening measuring 3 mm. in diameter, allowing communication between the chamber receiving the pulmonary veins and a second chamber lying beneath it (fig. 32). The latter had the characteristics of the left atrium

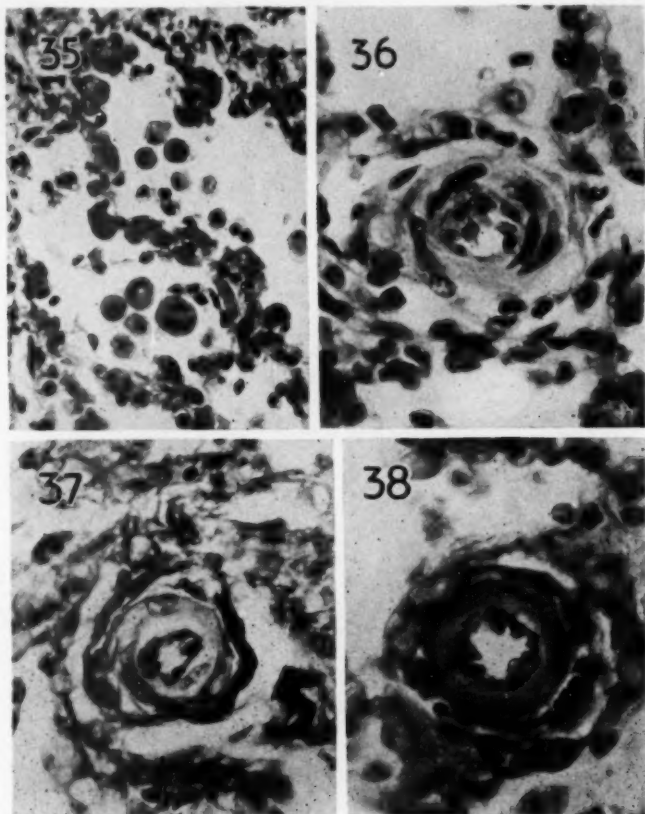


Fig. 35 (case 4).—Lung showing dilatation of alveolar capillaries to varicose proportions. (Hematoxylin and eosin;  $\times 380$ .)

Fig. 36 (case 4).—Pulmonary arteriole showing concentric cellular thickening of the wall causing luminal narrowing. (Hematoxylin and eosin;  $\times 600$ .)

Fig. 37 (case 4).—Pulmonary arteriole showing thickening of the wall and narrowing of the lumen as though by edema of the wall. (Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain;  $\times 600$ .)

Fig. 38 (case 4).—Intrapulmonary muscular artery showing a thick medial layer and prominent internal elastic membrane. (Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain;  $\times 740$ .)

in that it gave rise to the left auricular appendage and through its lower aspect communicated with the left ventricle through a normal mitral valve. The right atrium and the right ventricle

were dilated, and the wall of each was hypertrophied, the right ventricular wall measuring 6 mm. in thickness. The left ventricle appeared to be of normal size for an infant of this age. In view of the great size of the right ventricle the normal-sized left ventricle appeared to be an appendage of the right ventricle. All of the cardiac valves were normal. The coronary arteries arose from the aorta. The ductus arteriosus was closed. The venae cavae and the coronary sinus entered the right atrium in a normal fashion. The cardiac septums were closed. From the right atrium the foramen ovale was clearly visible. From the left side the valve of the foramen ovale was poorly outlined and presented in the medial wall of the accessory chamber. Microscopic examination of the wall of the left atrium and of the accessory chamber revealed in each a thick layer of cardiac muscle (figs. 33 and 34). The lining of the accessory chamber was similar to that of the atrium, both being composed of collagen and elastic tissue. The lining of the accessory chamber was thicker than that of the atrium proper.

Grossly the lungs were grayish purple and subcrepitant. Microscopic examination of the lungs revealed engorgement of capillaries (fig. 35). In some instances these vessels were strikingly tortuous. Scattered arterioles showed concentric cellular thickening of their walls, leading to severe degrees of luminal narrowing (fig. 36). In other instances small vessels, probably representing arterioles, showed narrow lumens and walls thickened by amorphous material, a picture possibly representing edema of the wall (fig. 37). The small muscular arteries showed medial hypertrophy and luminal narrowing, two characteristics which are abnormal for infants of the age of this patient (fig. 38).

Aside from a double ureter on the left side, the abdominal organs were normal, as was the brain.

#### COMMENT ON CASE 4

In 1949 Loeffler<sup>5</sup> reviewed the subject of the malformation represented in our case 4. He pointed out that three forms of the condition are recognizable. In the first there is no connection between the accessory chamber and the left atrium. This leads to death very soon after birth unless there is some mode of exit for the blood entering the accessory chamber.

Loeffler related that in Hagenauer's patient the accessory chamber communicated with the right atrium through a patent foramen ovale, while in Stoeber's<sup>6</sup> case some of the pulmonary venous drainage entered the right atrium. Our third case conforms to the first group of Loeffler.

In the second form there is a small opening between the accessory chamber and the left atrium. Our case 4 is an instance of this form and showed the usual phenomenon of death during infancy. The one exception from the point of view of longevity among cases with only a small opening between the accessory chamber and the left atrium is the case of Borst,<sup>7</sup> which concerned a woman who lived to be 38 years old.

In the third form of cor triatriatum the opening between the accessory chamber and the left atrium is large and so does not cause any functional disturbance. The patients with this form usually live to adult life. Loeffler's patient died at 70 years of age;<sup>5</sup> Griffith's,<sup>8</sup> at 48 years.

Loeffler reviewed the three hypotheses concerning the developmental basis of the malformation represented in our case 4. The first hypothesis considers that the septum which divides the accessory chamber from the left atrium is an overgrown and ectopic valve of the foramen ovale. The second hypothesis considers

5. Loeffler, E.: Unusual Malformation of the Left Atrium: Pulmonary Sinus, *Arch. Path.* **48**:371 (Nov.) 1949.

6. Stoeber, H.: *Virchows Arch. f. path. Anat.* **193**:252, 1908.

7. Borst: *Verhandl. d. deutsch. path. Gesellsch.* **9**:178, 1904-1905.

8. Griffith, T. W.: *J. Anat. & Physiol.* **37**:255, 1903.

that the malformation results from a misplacement of the common pulmonary vein, that the septum is the interatrial septum primum and that the opening in it is the interatrial ostium primum. Loeffler rejected each of the first two hypotheses on the basis that the atrial septum is usually properly formed. He accepted the third hypothesis, as do we. This is portrayed diagrammatically in figure 39. This hypothesis considers that the accessory chamber, into which the pulmonary veins open, is in reality a dilated common pulmonary vein and that the appearance of an accessory chamber results from failure of the common pulmonary vein to become incorporated into the dorsal wall of the left atrium as it normally should. The septum between the accessory chamber and the left atrium Loeffler considered to be formed by the

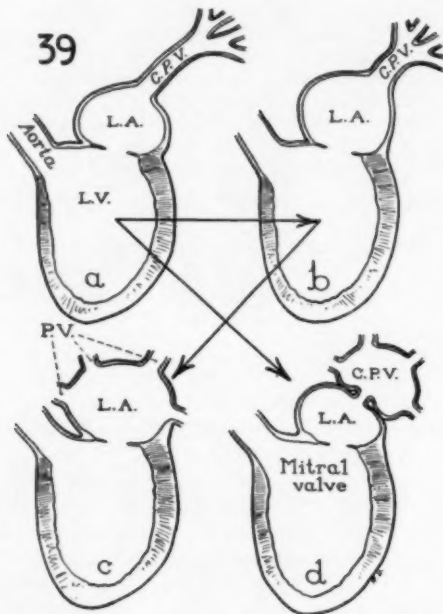


Fig. 39 (case 4).—Diagrammatic representation of the developmental basis of the malformation in case 4. Normally, as represented in *a*, the pulmonary veins connect with a common trunk, the common pulmonary vein (C.P.V.). As shown in *b* and *c*, the normal common pulmonary vein is progressively incorporated into the wall of the left atrium so that in the adult state each of the pulmonary veins communicates independently with the left atrium. The malformation in case 4 seems to have resulted from a failure of the common pulmonary vein to be incorporated into the left atrium, as represented in *d*. The stenotic connection between the common pulmonary vein and the left atrium was responsible for obstruction to emptying of the pulmonary veins and for dilatation of the common pulmonary vein to such a degree as to make it appear as an accessory cardiac chamber. The accessory chamber is therefore considered to be a dilated common pulmonary vein.

posterior wall of the primitive atrium. It would seem proper to consider that in reality it represents this part of the left atrium as well as the inferior wall of the expanded common pulmonary vein. The opening in the septum represents the junction of the embryonic common pulmonary vein with the sinoatrial portion of the heart. Primarily, therefore, the malformation represents a developmental arrest

at the stage when the sinoatrial region of the heart shows the evagination which is to become the common pulmonary vein. In addition, there are secondary effects of the obstruction that occurs between the common pulmonary vein and the left atrium. These effects include dilatation of the common pulmonary vein to give it the appearance of an accessory cardiac chamber, with hypertrophy of its wall and fibrosis of its lining, passive congestion of the lungs and pulmonary vascular changes like those observed in mitral stenosis, and right ventricular hypertrophy.

In keeping with this interpretation of the developmental basis of the malformation, Loeffler suggested that the malformation be designated as "heart with pulmonary sinus" rather than as "triatrial heart" (*cor triatriatum*). To us the designation "congenital stenosis of the common pulmonary vein" would seem most appropriate, although "*cor triatriatum*" is that most commonly employed in the few cases of this condition described in the literature.

The presence in our case 4, as in that of Potter and Ranson,<sup>9</sup> of the valve of the foramen ovale in the medial wall of the accessory chamber instead of in the medial wall of the true left atrium is difficult to explain. Only a suggestion can be offered. As the common pulmonary vein became distended incident to the narrow state of the outlet, it may have been evaginated into the left atrium and its outer wall secondarily fused with the atrial septum.

It is apparent that cases 3 and 4 are developmentally related. In the case of stenosis of the common pulmonary vein (case 4) it is assumed that the stenosis had occurred at a time subsequent to the obliteration of pulmonary venous-cardinal connections. Otherwise there might have developed a connection of the type seen in case 3. Contrariwise, it is possible that in case 4 the opening between the common pulmonary vein and the left atrium, though narrow, was adequate for the embryo and did not stimulate preservation of the connections normally lost. Case 3 represents a step in the direction of greater severity of pulmonary venous narrowing; in fact, the communication between the pulmonary veins and the left atrium was completely closed. As stated, the closure had occurred at a time prior to the normal obliteration of pulmonary venous-cardinal connections, thus allowing one of these to become elaborated into a vein of grossly recognizable proportions, which was an essential bridge in allowing flow of pulmonary venous blood.

The venous sinus receiving the pulmonary veins in case 3 is the structure analogous with the so-called accessory cardiac chamber of case 4.

Functionally the two cases are quite different. In case 4 the functional disturbance is very like that in mitral stenosis in that there simply was a barrier in the normal route of pulmonary venous blood. In the third case the functional disturbance resembled that of a two-chambered heart, with regard to the fact that all of the pulmonary venous blood was carried into the right atrium, where it mixed with all of the incoming systemic venous blood. The comparison of this malformation and the two-chambered heart can, of course, not be carried further, since in our case 3 the two ventricles were formed and it is likely that differential pressures existed between these two chambers.

The pulmonary congestion and occlusive vascular lesions and right ventricular hypertrophy observed in our case 4 appear related to the existing impaired venous return from the lungs. The pulmonary vascular lesions resemble those seen in other

9. Potter, P., and Ranson, S. W.: *J. Anat. & Physiol.* **39**:69, 1904.

conditions wherein there is an impediment to venous emptying of the lungs. Among these are acquired mitral stenosis,<sup>10</sup> mitral insufficiency<sup>11</sup> and acquired pulmonary venous stenosis.<sup>12</sup>

During early infancy the pulmonary vessels and particularly the muscular arteries of the lung have thick walls and narrow lumens. These features disappear as adult characteristics are taken on by these structures.<sup>13</sup> Realizing these facts and consequently the caution which must be exercised in calling the pulmonary vessels of an infant abnormally thick walled, we consider nevertheless that the structures exhibited by the pulmonary arterioles and small muscular arteries in case 4 are abnormal.

#### SUMMARY

This is a report of two cases of malformation of the pulmonary venous system.

In the first case the pulmonary veins entered a common sinus-like chamber which in turn communicated with the superior vena cava. This channel constituted the only avenue by which pulmonary venous blood could flow to the heart. From the lower end of the sinus-like chamber an atretic strand extended to the left atrium. This case is interpreted as one in which atresia of the lower portion of the common pulmonary vein of the embryo had occurred. The sinus-like chamber appears to represent the upper, patent portion of the common pulmonary vein. The connection between it and the superior vena cava is interpreted as a persistent communication between the splanchnic plexus of veins and the cardinal system, a connection which normally should disappear; in the presence of atresia of the lower portion of the common pulmonary vein this communication had persisted as a channel for the flow of pulmonary venous blood.

The second case was one in which the pulmonary veins emptied into a chamber lying dorsal and cephalad to the left atrium. This chamber communicated with the left atrium by means of a small opening. The accessory chamber is considered to be a dilated common pulmonary vein whose dilation resulted from its failure to become incorporated into the dorsal wall of the left atrium as it normally should. The functional effects of the narrow opening between the accessory chamber and the left atrium were similar to those produced by mitral stenosis. These resulted in pulmonary vascular changes similar to those observed in mitral stenosis, as well as in right ventricular hypertrophy. This case is classified as one of congenital stenosis of the lower portion of the common pulmonary vein.

10. Parker, F., Jr., and Weiss, S.: *Am. J. Path.* **12**:573, 1936. Larrabee, W. F.; Parker, R. L., and Edwards, J. E.: *Proc. Staff Meet., Mayo Clin.* **24**:316, 1949.

11. Becker, D. L.; Burchell, H. B., and Edwards, J. E.: *Circulation* **3**:230, 1951.

12. Edwards, J. E., and Burchell, H. B.: *Arch. Int. Med.*, to be published.

13. Civin, W. H., and Edwards, J. E.: *Arch. Path.* **51**:192 (Feb.) 1951.

## Case Reports

### CONGENITAL ANEURYSM OF THE JEJUNUM PRODUCING FATAL INTESTINAL HEMORRHAGE

THOMAS K. RATHMELL, M.D.

RICHARD J. HORWELL, M.D.

AND

JOSEPH P. GREELEY, M.D.

TRENTON, N. J.

**C**ONGENITAL aneurysm of the gastrointestinal tract is a rare lesion. Various descriptive adjectives, i. e., "saccular," "miliary," have been applied to it by several authors who found the lesion in the submucosa of the stomach. Its role in the production of fatal hemorrhage is not appreciated by members of the medical profession at large.

Sachs,<sup>1</sup> in 1892, reported an aneurysm situated on the tip of an elevation of the mucosa, about 4 mm. in size and 1.5 mm. in height, found in the lesser curvature of the stomach in a man of 79 years, who succumbed after severe gastric hemorrhage. Hirschfeld<sup>2</sup> found a pinhead elevation in the posterior gastric wall of a hemophilic patient of 38 years who had experienced 12 intense gastric bleedings prior to the final fatal hemorrhage. On histological study he found an aneurysm 3 mm. in size but was uncertain as to whether this ruptured artery would have produced death without the associated hemophilia. Two previous cases described by Gollard in 1884 were noted by Hirschfeld.

Hoelzer<sup>3</sup> reported a miliary aneurysm of the gastric mucosa with fatal hemorrhage in an 84 year old woman, and noted that the aorta and the cerebral vessels showed severe arteriosclerotic changes. There was also an associated aneurysm of the vertebral artery. Heuer<sup>4</sup> recently found such a lesion in the gastric wall. He commented on the surgical aspects of hemorrhage from peptic ulcer, as follows: Pathologically, the information regarding the source or sources of hemorrhage has been meager. At autopsy the pathologist has, generally speaking, failed to find an ulcer or other causes of hemorrhage. In one case the pathologist did not find even an erosion of the stomach and duodenum, but when the gastric artery was connected with a pressure bottle of salt solution a jet of fluid was projected from the mucosa. The area was excised and serial sections showed a small ruptured aneurysm covered by intact mucosa.

From the Departments of Pathology and Medicine, Mercer Hospital.

Dr. Rathmell is director of the laboratory, Dr. Horwell assistant in medicine and Dr. Greeley associate in pathology at Mercer Hospital (presently with Armed Forces Institute of Pathology).

1. Sachs, R.: Contribution to the Casuistry of Diseases of the Blood Vessels, *Deutsche med. Wchnschr.* **18**:443-447, 1892.

2. Hirschfeld, H.: Case of Fatal Gastric Bleeding Due to Miliary Aneurysm of an Artery of the Gastric Mucosa, *Berl. klin. Wchnschr.*, 1904; cited by Hoelzer.<sup>3</sup>

3. Hoelzer, H.: Fatal Hemorrhage from Miliary Aneurysm of Artery in Gastric Mucosa, *Zentralbl. f. Chir.* **63**:1996-1999 (Aug. 22) 1936.

4. Heuer, G. J., The Surgical Aspects of Hemorrhage from Peptic Ulcer, *New England J. Med.* **235**:777 (Nov. 28) 1946.

It is interesting to note that in a recent review entitled "Vascular Malformations and Vascular Tumors of the Gastro-Intestinal Tract," by Gentry, Dockerty and Clagett,<sup>5</sup> congenital aneurysm was not considered; neither was the lesion mentioned as a cause of gastrointestinal hemorrhage by Jones<sup>6</sup>; however, his discussion of the problem merits reference.

We wish to record the case of a patient seen in private practice by one of us (R. H.) because of sudden acute hemorrhage from the bowel without vomiting of blood at any time.

#### REPORT OF A CASE

A white woman of 29, married and the mother of two children, had been of excellent general health and appearance until the onset of a massive intestinal hemorrhage. Her past medical and family history were noncontributory other than that her father suffered from recurrent attacks of epistaxis of undetermined causes. Symptomatic medication brought no improvement, and within 72 hours the patient had more severe bleeding from the bowel and was hospitalized. This hemorrhage was so severe as to fill two hospital bed pans at a time. She was given blood transfusions and was thoroughly studied to exclude a blood dyscrasia, especially one of a purpuric character. Repeated transfusions failed to control the intestinal hemorrhage, and after consultation it was decided to subject the patient to an exploratory laparotomy. This was performed by Dr. Everitt B. Bearsto, chief of the surgical department.

Laparotomy showed the ileum to be distended with blood. However, no organic lesion or point of hemorrhage could be demonstrated throughout the entire length of the bowel, despite the fact that it was examined in segments with the aid of transmitted light, the luminal contents being excluded by manipulation.

On completion of the exploratory laparotomy, additional transfusions were administered, a total of 28 pints of blood being given in an attempt to control the massive hemorrhage. Despite this extensive use of blood transfusion, the patient died 36 hours after the surgical intervention.

*Necropsy.*—Postmortem examination was limited to the abdomen and was completed within two hours after death. The important anatomic findings consisted of necrosis of the parenchyma of the liver due to anoxia, superficial erosions of the gastric mucosa incident to intubation, incidental chronic peptic ulcers of the first part of the duodenum having a diameter of 0.7 cm., and massive fresh hemorrhage and blood clot within the lumen of the small intestine extending from the lower jejunum throughout the ileum and colon.

A minute search of the bowel mucosa revealed a mucosal polyp 0.5 cm. in diameter, projecting 0.5 cm. into the lumen of the colon, and in the second part of the jejunum a somewhat similar mass having a diameter of 0.5 cm. This mass presented a smooth, gray, glistening surface, was situated on the mesenteric border and projected into the lumen of the sectioned bowel for about 0.5 cm. Its consistency was that of a maraschino cherry. The massive hemorrhage present within the bowel lumen was noticeably present at this level.

After the material had been fixed in formaldehyde solution U. S. P. diluted 1:10, an area of discontinuity of the mucosal surface less than 1 mm. in diameter was visualized. Gross sectioning through this area and the central portion of the

5. Gentry, R. W.; Dockerty, M. B., and Clagett, O. T.: Vascular Malformations and Vascular Tumors of the Gastro-Intestinal Tract, Surg., Gynec. & Obst. **88**:281 (April) 1949.

6. Jones, C. M.: Diagnostic and Therapeutic Considerations of Gastro-Intestinal Bleeding, New England J. Med. **235**:773 (Nov. 28) 1946.

specimen showed a central space filled with blood (fig. 1). This was situated immediately beneath the mucosa and was comparatively thick walled (fig. 2).

Histological examination of sections of the specimen prepared with hematoxylin and eosin, modified Masson's trichrome and Verhoeff's elastica stains revealed that



Fig. 1.—Photograph of gross specimen (after formaldehyde fixation) magnified approximately 10 times. It shows a hemisection of the aneurysm as it projected from the jejunal mucosa. The indentation of the perimeter marks the point of rupture.

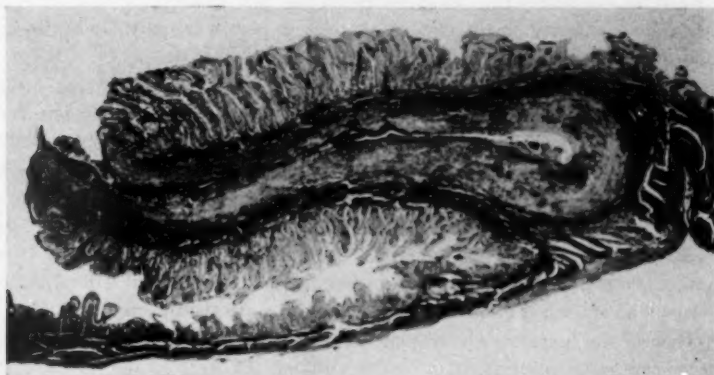


Fig. 2.—Photomicrograph of the submucosal lesion (approximately  $\times 10$ ) showing a thick-walled vascular structure, with the edge of abrupt mucosal defect indicating the point of rupture. The wall at this point is noticeably thin, owing to a defective medial coat.

the specimen consisted of a somewhat collapsed, thick-walled space, the wall of which could be divided into intima, media and adventitia and which contained blood. One edge had been perforated at a point where the media was thin, thus giving the contained blood access to the intestinal lumen. One edge of the lumen

showed thrombus formation with fibrosis and organization. Elastica fibers could be recognized under the hematoxylin and eosin stain by their wavy appearance and were shown to stain selectively with Verhoeff's elastica stain. The elastic fibers were diffusely distributed throughout the wall of the sac. Portions of internal elastic lamina were noted.

#### COMMENT

The differential diagnosis of the lesion was based on consideration and exclusion of the entities of polyp, telangiectasia, hemangioma and cavernous hemangioma, hemangioendothelioma, ulcerating neuroma and varicosity.

Histological observations indicated that the lesion was arterial. Etiological factors leading to aneurysmal formations were reviewed and differentiated as follows: atherosclerotic lesions—absence of histological changes of atherosclerosis in the wall of the sac and other body vessels; syphilitic involvement—no history of syphilis, negative serologic reactions; mycotic lesions—acute onset of illness and no histological evidence of acute inflammation in the sac wall; arteriovenous aneurysm—usually associated with trauma.

The lesion is therefore regarded as a ruptured congenital aneurysm of the jejunum. This opinion is based on the patient's age, the higher frequency of this entity in females, plus the clinical pattern of acute onset, massive hemorrhage and death, so frequently noted when these lesions occur intracerebrally, and the knowledge that elastica fibers may or may not be found in the sac wall of such structures when studied histologically, rupture of the aneurysm occurring because of defects in the medial coat.

Such a lesion had not been previously reported in the jejunum. This may be due to a laxity in examining the bowel lumen on the part of those doing postmortem studies and to a failure on the part of relatives to permit autopsy studies in cases of fatal intestinal hemorrhage.

It is suggested that if a radiopaque compound were injected into the superior mesenteric artery at the time of abdominal exploration and an immediate roentgenographic study made, this might aid in localizing lesions of the type described.

The case has been registered with the Armed Forces Institute of Pathology as Accession No. 290683, where there was some difference of opinion among the staff as to whether the vascular channel described was a vein or an artery.<sup>7</sup> It is our opinion, on the basis of the differential diagnosis presented, that it is an arterial channel. The patient at one time filled three bed pans with blood within an hour. Clinicians who studied the case felt the bleeding was arterial in origin.

Review of the literature shows that six congenital aneurysms have been noted in the human gastrointestinal tract by five authors. All were located in the gastric mucosa. Previous to our report, congenital aneurysms of the human jejunum had not been described.

Congenital aneurysm must be considered in the differential diagnosis of gastrointestinal hemorrhage. More adequate procedures, either roentgenological or surgical, should be developed for its identification and control.

7. Dart, R. O., director, Armed Forces Institute of Pathology: Personal communication to the authors.

## SUMMARY

We have reported a case of congenital aneurysm of the jejunum with rupture and fatal gastrointestinal hemorrhage. Literature pertaining to small aneurysms of the gastrointestinal tract has been reviewed and a suggestion presented which may aid in future diagnosis of this lesion. This pathological entity provides an explanation for some cases of massive gastrointestinal hemorrhage of undetermined origin.<sup>8</sup>

The illustrations were prepared by Harold A. Thomas, Laboratory of Pathology, Presbyterian Hospital, Philadelphia.

8. Jankelson, I. R., and Milner, L. R.: Massive Upper Digestive Tract Hemorrhage of Undetermined Origin, *J. A. M. A.* **145**:17-21 (Jan. 6) 1951.

## MALIGNANT EPENDYMOMA INFILTRATING THE SCALP

IRWIN FEIGIN, M.D.

AND

BRUNO W. VOLK, M.D.

BROOKLYN

**T**UMORS of the glioma group remain a source of confusion in that their classification is frequently difficult and their properties often differ from the general properties ascribed to tumors of other groups. In general, the most malignant gliomas very rarely metastasize or infiltrate into non-neurogenic tissues, although their rapid growth and cellular atypism would lead to that expectation. The tumor to be described is atypical in that it infiltrated into the extracranial tissues through a bone flap to form a bulky subcutaneous mass. Its classification has been a source of controversy, but recent suggestions for simplifying the classification of some of these tumors are particularly applicable in this case, and the tumor is considered to be a cancerous ependymoma.

### CLINICAL DATA

A 29 year old man was admitted to the Neurological Institute, New York, because of bitemporal headache, diplopia, nausea and one episode of vomiting over a seven week period. Neurological examination revealed weakness of the right upper and lower extremities, 3 plus papilledema bilaterally, and diplopia due to paresis of the right external rectus muscle. There was slight left hyperreflexia but no abnormal reflexes on either side. Ventriculography revealed that the right lateral ventricle was displaced to the left. A diagnosis of right frontal neoplasm was made, and craniotomy was performed. A cystic tumor was found and partially removed after evacuation of the cyst. Postoperatively, radiation therapy was instituted consisting of 13 treatments of 200 roentgens (r) each. All symptoms and signs disappeared except for the papilledema, which persisted, though to a lesser degree.

The patient did relatively well for eight months. Then there was a recurrence of symptoms. This included bitemporal headache, diplopia, dizziness, olfactory and gustatory hallucinations, and nausea. The patient was readmitted to the Neurological Institute one month after the recurrence of symptoms. Physical examination revealed the bone flap to be elevated, and the decompression area to be bulging and tense. There were generalized hyperreflexia, greater on the left, very slight hypertonia and weakness of the left upper extremity, nystagmus on right lateral gaze, paresis of the right external rectus muscle, left central facial weakness and 3 plus bilateral papilledema. Craniotomy was performed again, and additional portions of the tumor were removed. Radiation therapy was instituted directly to the neoplastic area through the open wound. Postoperatively there was no relief of symptoms, although the headaches became less severe.

Two months later the patient was readmitted because of generalized convulsions and vomiting. The bone flap was found to be elevated, and the decompression area was bulging. Slight weakness of the left upper and lower extremities and left central facial weakness persisted. Secondary optic atrophy was thought to be present. The patient was discharged unimproved.

From the Division of Laboratories, Jewish Sanitarium and Hospital for Chronic Diseases, Brooklyn, and the Neurological Institute, and the Neuropathology Unit of the Department of Pathology, Columbia University, New York.

Six weeks later he was readmitted with complaints of severe headache, frequent vomiting, diplopia and weakness of all extremities. Physical examination revealed in addition hypesthesia, hypalgesia, decreased vibratory sensation, hyperreflexia, a Babinski reflex, and central facial paresis, all on the left, paresis of the right external rectus muscle, elevation of the frontal bone and bulging of the decompression area. Complete right frontal lobectomy was performed at this time. Postoperatively the patient was somewhat better, although most signs and symptoms persisted with decreased intensity. There was recurrence of generalized convulsions four months later, associated with complete left hemiplegia.

A loss of visual acuity became apparent, and the patient was admitted to the Jewish Sanitarium and Hospital for Chronic Diseases. The most striking change was the presence of a bulging mass involving the greater portion of the right side of the head (fig. 1 *A*). At this time the patient was completely blind. Neurological examination revealed complete left hemiplegia, left central facial weakness and paresis of the right external rectus muscle. The patient's general condition deteriorated, pneumonia supervened and death occurred one month later. The total course was 24 months in duration.

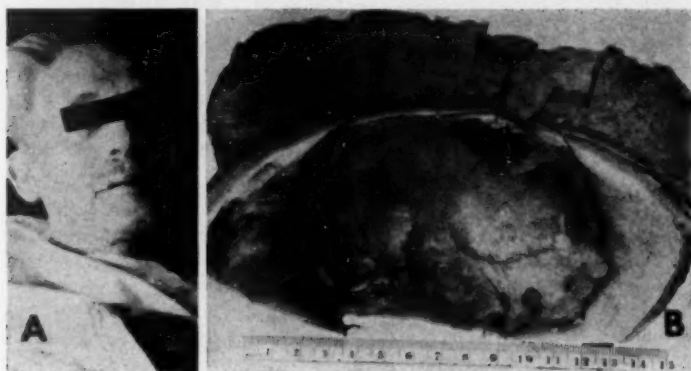


Fig. 1.—*A*, the patient approximately one month before death. *B*, the calvarium, showing the extracranial mass of neoplasm and the area of craniotomy.

#### POSTMORTEM OBSERVATIONS

The body appeared fairly well nourished. Decubitus ulcers were present over the sacrum and the trochanters of the femurs. The lungs revealed evidence of diffuse terminal lobular pneumonia. The other viscera showed no significant changes.

There was a large protuberant mass involving the major portion of the vault of the right side of the skull. This mass protruded approximately 45 mm. above the level of the calvarium (fig. 1 *B*). It was somewhat irregularly lobulated and firm in consistency. The scalp was closely adherent to the underlying tumor. Dissection of the scalp revealed the protuberant growth to consist of a moderately soft, friable, somewhat variegated mass of gray-pink tissue. This tissue was not distinctly circumscribed but infiltrated diffusely in the tissue layers between the bone and the skin, being firmly attached to both. On removal of the calvarium, the right cerebral hemisphere was found to be firmly adherent to the overlying bone around the margins of a large bone flap in the right temporal area. This bone

flap measured 12 by 10 cm. At its lower margin there was a defect in the continuity of the bone of the calvarium measuring approximately 6 cm. in diameter. Interrupted silk sutures were visible at its margins. Neoplastic tissue was seen penetrating through the tissues between the bone flap and the remainder of the calvarium throughout its circumference, and through the region of the decompression area. In addition, an area of direct continuity between the intracranial and extracranial tumor tissue, 15 mm. in diameter, was noted at the superoanterior margin of the bone flap. It was infiltrating and destroying the remaining parenchyma of the left frontal lobe. The posterior portion of the ventricular system was not dilated or deformed. In some areas a granular ependymitis was present. The cerebellum and the brain stem revealed no gross abnormalities. The major portion of the right frontal lobe was absent, the tissues remaining being represented by a shell of connective tissue enclosing an irregular cystic space. In places they were quite friable and contained nodules of neoplastic tissue resembling that in the scalp. The largest nodule remaining intracranially measured 6 cm. in diameter. It was somewhat softer than the tumor external to the cranium and was slightly more friable. It was firmly attached to the dura. The tumor extended across the midline through the corpus callosum, forming a nodule approximately 2 cm. in diameter in that structure. Neoplastic tissue was found in the left superior frontal gyrus anteriorly, where it was continuous with the large intracranial neoplastic mass mentioned above.

#### MICROSCOPIC STUDIES

Except for minor differences to be described, the histological appearance was essentially the same in the tissue removed in the various operative procedures and at autopsy. The individual cells for the most part were small, polygonal or irregularly shaped, with round or oval, moderately or deeply chromatic nuclei. There was an obvious tendency for the cells to be arranged circumferentially about blood vessels, with the nuclei separated from the vessel by a clear cytoplasmic zone (fig. 2 *D*). Many true rosettes were present (fig. 2 *C*) composed of columnar cells arranged in a circular fashion about a lumen. Broad bands of cells with parallel edges were seen, between which a central vessel could be noted in some but not all instances (fig. 2 *B*). Numerous blood vessels were present, many of which showed considerable hyperplasia of endothelial cells, with lesser proliferation of fibroblasts in the adventitia. Bands of connective tissue were found separating islands of neoplastic cells. Sections from the scalp revealed tumor infiltration of the subcutaneous tissue and of the corium similar to that found intracranially (fig. 2 *A*).

There were minor differences in the appearance of the tumor in the various specimens removed at operations and at autopsy. The tissue removed at the first operation had many mitotic figures in the neoplastic cells, a feature much less prominent in subsequent specimens. The quantity of fibroblastic connective tissue was minimal in the first specimen but significantly greater in the tumor removed at a later date. Many foci of calcification were present in the tissues removed at the last surgical procedure but not in the other specimens. The neoplastic tissue removed at autopsy showed very few differentiated areas, most of the tissue being composed of small dark cells, irregularly distributed. A few well formed rosettes were noted.

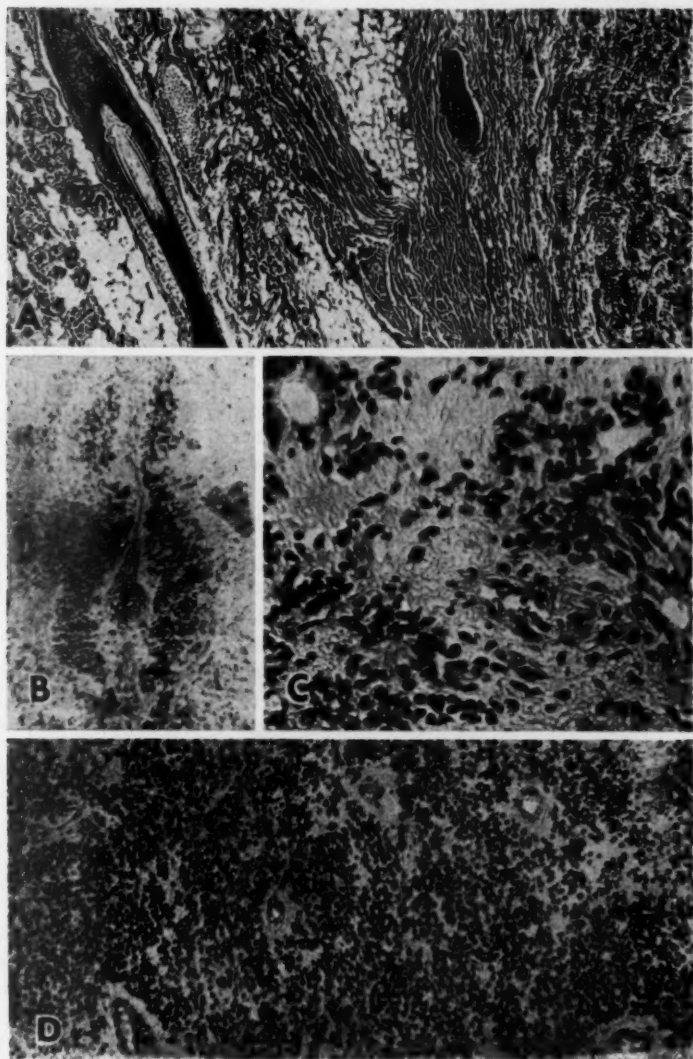


Fig. 2.—*A*, tumor cells infiltrating the skin of the scalp;  $\times 50$ . *B*, tumor cells arranged in bands which assume the appearance of a medulloepithelioma;  $\times 85$ . *C*, tumor cells in rosette formation as seen in neuroepithelioma or ependymoma;  $\times 170$ . *D*, tumor cells in perivascular arrangement as seen in ependymoma;  $\times 100$ .

Microscopic examination of the lungs confirmed the impression of a lobular pneumonia. Sections from the remaining areas showed no conspicuous changes.

#### COMMENT

This neoplasm is of interest in that it penetrated through a decompression area in the skull and around the margins of a bone flap to form a large bulky mass beneath the scalp (fig. 1), infiltrating the fibrous connective tissue of the scalp and the corium of the skin (fig. 2A).

In general, the malignant tumors of the glioma group differ in some respects from malignant neoplasms arising in other organs. Although metastases along the subarachnoid space are relatively frequent in some, notably the medulloblastoma, a glioma metastasizing to tissues outside the central nervous system is so rare as to be considered a curiosity. Bailey and Cushing<sup>1</sup> considered glioblastoma multiforme to be the only glioma known to invade extracranial tissues through a decompression area, although they stated that this is very unusual. They stated, further, that distant metastases do not occur. Freeman and Zimmerman<sup>2</sup> reported that they had successfully transplanted gliomas into the anterior chamber of the eye in experimental animals. They considered this to indicate that the glioma has the potentiality of growth in tissues outside the central nervous system. Distant metastases of these transplanted tumors did not occur.

Those who assume that these tumors have the capacity of growth in extracranial locations attempt to explain the absence of distant metastases by the effectiveness of the physiological blood-brain barrier, whatever its nature. However, when necrosis involving blood vessels occurs, as it does quite commonly in cases of glioblastoma multiforme, or when a malignant growth is found in an extracranial position after experimental transplantation as reported by Freeman and Zimmerman,<sup>2</sup> or after having infiltrated directly through a decompression area, as in the case being reported, and in those cases referred to by Bailey and Cushing,<sup>1</sup> no blood-brain barrier is present. Yet distant metastases do not occur. This problem remains to be resolved.

Recent studies by Kernohan and associates<sup>3</sup> have tended to simplify the classification of tumors of the glioma group originally suggested by Bailey and Cushing,<sup>1</sup> a simplification most usefully applicable in the case being reported. At different times and by different pathologists a tumor such as this one has been considered a medulloepithelioma, a medulloblastoma, a neuroepithelioma and an ependymoma.

A diagnosis of medulloepithelioma is suggested by the presence of bands of cells arranged to resemble the embryonic medullary plate (fig. 2B). Such bands of cells appearing in a rapidly growing tumor may actually represent cells which are relatively preserved because they are near a nutrient blood vessel, more distant cells having undergone necrosis (fig. 2B). Other similar cell formations were

1. Bailey, P., and Cushing, H.: *A Classification of the Tumors of the Glioma Group and Histogenetic Basis with a Correlated Study of Prognosis*, Philadelphia, J. B. Lippincott Company, 1926.

2. Freeman, D., and Zimmerman, H. M.: *Cancer Research* 4:273, 1944.

3. Mabon, R. F.; Svien, H. J.; Kernohan, J. W., and Craig, W. McK.: *Proc. Staff Meet., Mayo Clin.* 24:65, 1949. Kernohan, J. W.; Mabon, R. F.; Svien, H. J., and Adson, A. W.: *Ibid.* 24:71, 1949.

present in which this relationship was less apparent. If our interpretation should be correct, the resemblance to the medullary plate would be merely fortuitous. Kernohan and associates<sup>3</sup> studied the only four examples of this type of tumor diagnosed at the Mayo Clinic up to 1949 and reclassified three of them as ependymomas and one as a medulloblastoma. An example of medulloepithelioma illustrated in Bailey and Cushing's text demonstrates a perivascular arrangement of cells similar to the basic cell arrangement of the ependymoma.

A diagnosis of cerebral medulloblastoma was made on those occasions when sections of the tumor revealed it to consist entirely of small dark undifferentiated cells. The major portion of the tumor was composed of such cells. The photographs were chosen to illustrate those portions of the tumor which are more differentiated, and therefore serve to identify it. The presence of such differentiated areas even when they comprise a small portion of the tumor, serves to make a diagnosis of medulloblastoma untenable.

A diagnosis of neuroepithelioma is based on the presence of numerous well formed rosettes (fig. 2C). These consist of a tubular structure lined by columnar cells and are said to resemble the primitive neural tube. There was no example of neuroepithelioma in the series described by Bailey and Cushing.<sup>1</sup>

This tumor most usefully may be classified in the ependymoma group. The most characteristic feature of the ependymoma is the tendency of the cells to arrange themselves in a circular fashion around blood vessels with the nuclei distant to the vessel and the cell bodies near the vessel (fig. 2D). The cell bodies tend to form a clear pale area surrounding the vessel so that a structure resembling a rosette is formed. Such a structure is sometimes called a pseudorosette and differs from a true rosette in not having an actual central cavity. It has come to be realized that many tumors which show the basic clinical and pathological features of the ependymoma do contain true rosettes as well as the pseudorosettes just described. Some standard texts include the rosette in their description of the ependymoma,<sup>4</sup> while others do not. Kernohan<sup>3</sup> discards the term "neuroepithelioma," reclassifying most such tumors as ependymomas.

That this simplified classification is applicable in this case is readily apparent. Some portions of the tumor showing the perivascular arrangement of cells (fig. 2D) are readily identified as characteristic of ependymoma, as is true of the rosettes.

#### SUMMARY

A rapidly growing malignant tumor of the glioma group is described which proved fatal in a two year period despite repeated operations and intensive radiotherapy. The tumor infiltrated extracranial tissues through the margins of a bone flap in a manner resembling that of non-neurogenic cancers but did not metastasize. It is most usefully classified as a malignant ependymoma.

4. Lichtenstein, B. W.: *A Textbook of Neuropathology*, Philadelphia, W. B. Saunders Company, 1949. Hassin, G. B.: *Histopathology of the Peripheral and Central Nervous Systems*, Baltimore, William Wood & Company, 1933.

## Notes and News

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**DeCoursey Made Brigadier General.**—Colonel Elbert DeCoursey, former Commandant of the Army Medical Service Research and Graduate School, Army Medical Center, Washington, D. C., and director of Armed Forces Institute of Pathology, since last August, has been advanced to the rank of brigadier general. During World War II he was appointed a member of the Joint Commission for the Investigation of the effects of the atomic bomb in Japan in 1945. He also served as consultant to the Division of Biology and Medicine of the Atomic Energy Commission.

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## Books Received

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**VERDAUUNGS- UND STOFFWECHSEL- KRANKHEITEN.** By Prof. Dr. Max Bürger, director of the Medizinischen Universitäts Klinik Leipzig. Pp. 440, with 123 illustrations (43 in color) and 73 tables. Price, paper, 55 German marks; cloth, 59 German marks. Ferdinand Enke Verlag, Hasenbergsteige 3, Stuttgart, 1951.

**ATLAS DER NORMALEN HISTOLOGIE UND MIKROSKOPISCHEN ANATOMIE DES MENSCHEN.** By E. von Herrath, professor of anatomy in Freien Universität, Berlin, and director of Anatomischen Anstalt, and S. Abramow, formerly professor in the universities of Moscow and Sofia. Pp. 140, with 398 illustrations, most of them colored. Price \$11.45. Georg Thieme Verlag, Diemershaldenstrasse 47 (14a) Stuttgart, O; (Grune & Stratton, Inc., 381 Fourth Ave., New York 16), 1950.

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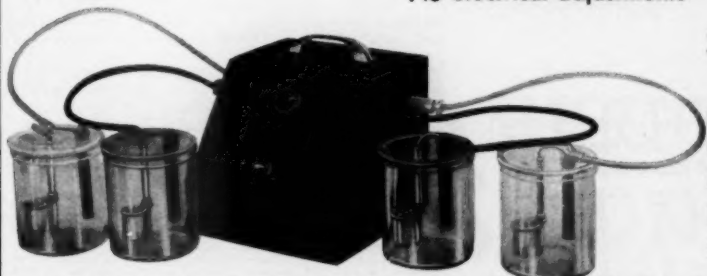
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there's the answer to every laboratory filing need in these interchangeable stocking units —

- **\*1" MICROSLIDE DRAWERS**  
(for spaced or storage filing)
- **\*2" SLIDE OR TRANSPARENCY DRAWERS**  
(for spaced or storage filing)
- **\*4" DRAWERS**  
(for 3 1/4" x 4 1/4" slides or index cards. Technilume drawer for built-in illumination optional)
- **SHELF**  
(for resting trays or drawers in use)
- **FLAT-FILING CABINET FOR MICROSLIDES**  
(slides lie flat in trays; dry quickly; are visible at a glance)
- **FLAT-FILING UNIT FOR TRANSPARENCIES**  
(2" x 2" or 3 1/2" x 4 1/4" for filing and instant viewing)
- **ILLUMINATION UNIT**  
(used in conjunction with Transparency Flat-Filing for viewing)
- **PARAFFIN BLOCK FILE**  
(shallow drawers, with vent-sized knock-down cardboard containers)

\*Single cabinets can comprise variable assemblies of any of these drawers, thanks to the Technicon Lab-aid unit track design.



Everything about a Technicon Lab-aid file is designed to speed the work and improve filing efficiency. For example, the unique (patented) spring slide-spacers. Holding slides upright with plenty of air-space for quicker drying, they're still supple enough to spread like this, so you can easily file or find a slide. If you want more capacity for close-packed, permanent storage, you simply remove the spacer, which gives you room for approximately 6500 slides in a single 19" x 19" x 5" unit — up to 45% more capacity.

To find out more about this efficient system for laboratory filing, send the coupon below.

**technicon** *Lab-aid* filing system



THE TECHNICON COMPANY  
215 East 149th St., New York 51, N. Y.

Send me particulars of the "LAB-AID" filing system.

Name

Address

City  State